The Microscopic Diagnosis of Tropical Diseases

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PREFACE

This book has been developed from our previous brochure entitled "The Microscopic and Chemical Diagnosis of Tropical Diseases" published more than a decade ago In this present edition the emphasis is laid on microscopy Even in the previous edition it was only possible to include the simplest chemical diagnostic methods, and since then chemical and serological diagnostic techniques have developed so tremendously that it is impossible to fit them into this new edition We have, therefore, in this edition concentrated on that aspect of laboratory diagnosis which proved, in the old edition, to be of special interest to the physician in the tropics, namely, microscopical diagnosis Several virus diseases, whose causative organisms may be seen under the microscope, and which are of special interest to the tropical practitioner, are also dealt with

This present book, then, is intended to serve three purposes

1 To give the physician a short survey of microscopic technique impor tant for the diagnosis of infectious diseases
2 To amplify the illustrations by indicating the salient points whic should be studied when examining microscopic preparations
3 To outline a few virus diseases, the causative organisms of which m be seen under the microscope notwithstanding the fact that, to general practitioner, such methods of demonstration may be too co plicated

The limited space available makes it necessary to restrict the amount of technical information given on microscopical diagnosis and the description of the corresponding diseases. Not only purely tropical diseases are discussed here, however, but also some ubiquitous diseases which are of increased importance in the tropics.

Wherever in this book suggestions are made regarding therapy, no attempt is made to present an exhaustive summary. Those products are only named when either they have been developed by us especially for the treatment of protozoal and helminthic diseases, or when they appeared to be particularly indicated.

It is our hope that this new edition will be appreciated as much as the old, and not only appreciated by the many friends of that edition, but also by new readers, thus gaining new friends for us and our endeavours in the field of medicine.

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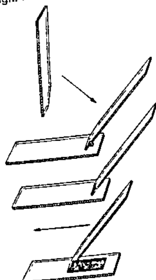
General methods in microscopy

Blood films

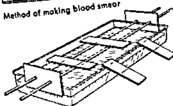
The demonstration of the causative organisms of tropical diseases in blood can be made either in wet film or fixed and stained preparations. For the examination of wet blood films by normal or dark field microscopy, one drop of blood is spread out between the microscope slide and the cover glass, so that the erythrocytes are only one layer thick. Blood may be obtained from the nail-bed, ball of the finger, or from the lobe of the ear The skin should previously be cleaned with ether or alcohol, and rendered hyperaemic. The skin may be punctured with a straight triangular suture needle or, if this is not available, a hypodermic needle, or even a pen nib, one point of which has been broken off. A spring-loaded puncture device is sometimes used, but suffers from the disadvantage that it is difficult to sterilise

Obtain

Light pressure may be applied in order to assist the flow of blood, the second or third drop of which should be used for examination. The drop may either be set directly on the slide or taken onto the edge of a cover slip In either case the slide must be spotlessly clean and fat-free The drop should then be allowe to spread out either along the edg of another slide (or of the same cov slip) held at an angle of 40°—5 Care should be taken to pull blood from one end of the slide to other and not to push it. The should be allowed to dry in the with the help of a fan, and then



Method of making blood smear



Staining rack

Fixation of smear with methyl alcohol (1—5 minutes) or with absolute ethyl alcohol (15—20 minutes) The film may be fixed on a staining rack, in a covered Petri dish, or in a staining jar. The fixed film may be dried in the air after the alcohol has been poured off, or pressed dry between sheets of filter paper.

Field's staining method Field's stain is suitable for the staining of thin blood films for differential counting, and gives, in the majority of cases, a better result than either Giemsa or Leishman since no deposit of stain occurs on the slide. Although in expert hands Giemsa staining enables a finer definition of structural details to be achieved, yet it is only very seldom that this amount of detail is required in practice. In the hands of the average technician, Field's staining for routine use produces results superior to those obtained with Giemsa stain

This staining method is suitable for either thick or thin films.

Solution A

Methylene blue	1.3 g.
Disodium hydrogen phosphate anhyd. (or 12.6 g of cryst.)	5.0 g

Dissolve above in 50 cc water, bring to boil and evaporate almost to dryness on water-bath. Then add potassium dihydrogen phosphate, anhydrous, 6.25 g (or 8.0 g. of crystalline salt). Add 500 cc. warm distilled water, stir and set aside for 24 hours Filter before use.

Solution B

Eosin	1.3 g.
Disodium hydrogen phosphate anhyd.	5.0 g.
Potassium dihydrogen phosphate anhyd.	6.25 g.
Distilled water	500 cc.

Dissolve phosphate in freshly boiled distilled water, then add eosin Set aside for 24 hours, filter before use

The solution may be kept in staining jars on the bench, and the same solution used continuously for hundreds of slides. In the tropics a few drops of chloroform will prevent mould growth occurring. The eosin solution should be renewed when it becomes greenish from the carry-over of methylene blue.

Method of Staining

Thin films

1. Fix slide with a few drops of methyl alcohol, 30 seconds to 1 minute.
2 Wash off methyl alcohol.
3 Dip slide into Solution A for 1—5 seconds.
4 Rinse immediately by waving gently in water
5. Dip slide into Solution B for 1—2 seconds.
6 Rinse immediately by waving gently in water
7. Re-dip slide into Solution A for 1—2 seconds only (This second dip is not always necessary.)
8. Rinse in water and blot dry

The relative times may require a slight adjustment depending upon the degree of polychroming of the methylene blue

Thick films

1. Dehaemoglobinize in water
2. Dip in 'A' for 3 seconds
3 Rinse very gently for a second or two
4 Dip in 'B' for 5 seconds
5. Rinse again very gently until stain ceases to flow away
6 Place vertically against a rack to dry.

Giemsa staining for blood and tissue smears

A most important stain for use in the tropics is the Giemsa stain which follows the principle of Romanowsky staining It is specially suitable for the demonstration of malaria parasites, trypanosomes and leishmania Giemsa stock solution, as it is bought, should be protected from light and kept tightly closed and stored in a cool place, as otherwise it tends to deposit. For staining, this stock solution should be diluted, and it should be diluted freshly every time it is required. It is best to prepare small quantities of the diluted stain when wanted For this purpose 1—2 drops of the Giemsa stock solution should be diluted with 1 cc. of water. For larger quantities 3.5—10 cc of stock solution (pipetted) should be diluted with 100 cc of water, the quantity of stock stain varying according to the type of staining to be done and the manufacturer of the stain The stock solution should be pipetted into a large diameter measuring cylinder (at least 3 cm. in diameter) and the necessary amount of water then added Large quantities of stain should be gently stirred

with a pipette or with a glass rod. Vigorous shaking or swirling will easily precipitate the dye-stuff. The diluted stain should be used immediately.

The water used for diluting the stock stain should be neutral or weakly alkaline. This often causes difficulties in the tropics. It is preferable before using tap, rain or distilled water to remove the carbon dioxide by boiling for a short time. It is, however, even better to buffer the water by adding 0.49 g. of primary potassium phosphate and 1.14 g of secondary sodium phosphate to one litre of boiled dist. water.

The slides should be placed on the staining rack without touching one another, and the staining solution should then be poured on them. They should be stained for 40—45 minutes. If, during this period, the staining solution precipitates out, it must be replaced by new. After staining, the slide should be washed in rapidly running water, or the stain may be washed off by carefully pouring water from a beaker onto one corner of the slide The slide should then be left upright to dry. For this purpose a good slide-board may be made from a block of wood 40 cm long, $5^{1}/_{2}$ cm. broad and $2^{1}/_{2}$ cm high, the length of which contains parallel slots $1^{1}/_{2}$ cm. deep cut at an angle of 35°—45° separated by a distance of $2^{1}/_{2}$ cm Twelve to 15 slides may be placed on end in these slots and allowed to dry in the air or with the help of an electric fan.

For the demonstration of flagella and many delicate spirochaetes, it is recommended to heat the stain in a beaker until it steams. This hot solution is then used to stain, left on for 15 minutes, and then replaced with a second, hot, stain solution for another 30 minutes. It is also possible — as with ZN staining — to heat the slide with the cold stain on it, provided that the heating is gentle. With this hot staining, the flagella and spirochaetes stain more intensely than with the cold method.

The dried slides should be examined direct under oil immersion, no cover glass is necessary. If it is desired to keep the preparation as a permanent specimen, then it should be mounted in Caedax (neutral mounting medium) after one has removed the immersion oil with xylene. Smears can be made of other body fluids, biopsy material, secretions, etc., and treated in the same manner as blood films. Biopsy material can be "smeared" on the slide and the expressed cells and tissue fluids treated as described above.

American pathologists use Wright's stain as a routine method Although the method is more rapid, the final staining is neither as intensive nor as permanent as a Giemsa stained preparation The technique is as follows: The undiluted stain is poured onto the slide and allowed to stain for 3—5 minutes The methyl alcohol contained in the preparation fixes the film. Buffered distilled water is then poured onto the slide until the surface of the liquid takes a metallic green tint. This is allowed to stain for 5—20 minutes, according to the material being examined, and then is washed in running distilled water It is dried and then immediately examined. The optimal staining time for the various protozoa depends upon the various batches of stain as well as the organism in question. Enough of the undiluted stain should be used initially to avoid too great a dilution when the distilled water is added Washing of the stained specimen with distilled water is important, to ensure that particles which have deposited from the solution during the staining will be washed off the slide, and also to differentiate the cytoplasm of the cells and the protozoal structures present

Wright's stain

The thick-drop technique is a method by which a concentration of the parasites is achieved It is of special value when parasites are few, and often permits a positive diagnosis when an ordinary thin film gives negative results By means of the puncture needle, or by direct transfer, 2—3 small drops of blood are placed on the slide and spread out in a circle of 1—1.5 cm. diameter. (It must be thin enough just to see the hands of a wrist watch through). It is then allowed to dry thoroughly, this usually requires 30 minutes to 2 hours depending on the temperature, humidity and thickness of the preparation. Thorough drying is very necessary because the drop, not being fixed to the slide, is easily washed off during the subsequent staining Even if this should happen it is still frequently possible to examine the edge of the drop which generally remains stuck. During the initial drying, the drop should be protected against dust and insects (flies and ants) — a bell-jar or even Petri dish cover is usually suitable.

Method for thick drop film

When the thick drop is dry, it is stained without previous fixing. When stained with diluted Giemsa, the water used to dilute the stain will automatically dehaemoglobinize the erythrocytes, which do not then take the stain, only the leucocytes and parasites staining with this tech-

Staining of thick drop

nique. With Wright's stain the thick drop must be dehaemoglobinized with distilled water prior to staining.

After staining, the slide is carefully washed by passing gently to and fro in a beaker of distilled water. This removes the stain and any deposited particles. Overstaining is corrected by placing in distilled water, this then enables the parasites to be clearly differentiated. In the thick drop, the parasites are concentrated in a small space, but the staining of the parasites is often not good enough to allow of a differential diagnosis, in malaria for example. Should such a diagnosis be required it is necessary to prepare and stain a thin film. See also Field's stain p. 4.

Giemsa long staining method

Giemsa staining by the long method is generally employed for the *staining of tissue sections in histology, and for the demonstration of certain* viruses in smears from organs, etc. The latter preparations should be allowed to dry, fixed by a brief immersion in methyl alcohol and stained as follows· place the slide in the staining jar containing the diluted stain (5 cc Giemsa stock solution → 100 cc. buffered water) for 16—24 hours at 20—22° C. Carefully pour off the stain to avoid the metallic skin depositing on the slide, and then wash in running water. Differentiate by dipping in and out of 96 % alcohol and then immediately follow with a brief rewashing The slide is then placed for 2 minutes in a mordant of 1 g orange G plus 5 g. tannin → 100 cc. distilled water. (This mordant may be preserved by the addition of one or two *crystals of phenol; it may be used repeatedly). The slide, after the mor*danting, should be washed and stood to dry.

The staining of paraffin sections by the long method is very tedious, and the tropical physician is hardly likely to be able to carry it out. The method is given here, however, since most of the virus preparations given in the plates have been stained by this method.

Remove paraffin from section with xylene, and bring into buffer solution through alcohol stages Then into Giemsa (2.5 cc. Giemsa stock → 100 cc. buffered distilled water at pH 6 8—7 0 + 0.5 cc. of a 1 % acetic acid solution) and stain for the same time as above Wash several times in water, differentiate briefly in water slightly acidified with acetic acid; wash again in distilled water. Differentiate in acetone until no further colour comes out of *the preparation. Then take through acetone-xylene mixtures (3:1), 2:2, and* 1:3) into pure xylene, and finally embed in Caedax.

Demonstration of filaria in wet preparations

For the demonstration of microfilariae in the blood, a wet preparation

is suitable. A drop of fresh blood is spread between a cover glass and slide, and the microfilariae are easily detectable under low power by means of their rapid movement. A species identification of the microfilaria is, however, not possible by this method The following method has been developed for this purpose and it is similar to the thick drop technique used for the demonstration of blood protozoa.

On a clean, fat-free slide, 2—3 large drops of blood are spread out to an area of 2—3 sq. cm. The slide is dried as rapidly as possible It is dehaemoglobinized by placing the slide face downwards in a Petri dish containing distilled water. The slide should be placed so that one end is resting on one edge of the Petri dish. The dehaemoglobinized blood drop is allowed to dry and is then fixed in absolute alcohol for at least 10 minutes The usual method of staining is that of Delafield described on page 13 of this booklet. Also suitable is the following method of Hansen. For this the following staining solution is required *Stained Preparations*

(a) 1 g haematoxylin is dissolved in 10 cc of absolute alcohol
(b) 20 g potassium alum is dissolved in 200 cc. of warm distilled water and filtered when cold.
(c) 1 g potassium permanganate is dissolved in 16 cc distilled water

On the following day (a) and (b) are mixed in a porcelain dish, stirred continuously, whilst exactly 3 c.c. (pipetted) of the solution (c) are slowly run in This mixture is then heated under continuous stirring until it boils. It should be allowed to boil for 30 seconds to 1 minute (maximum), rapidly cooled and then filtered The addition of potassium permanganate converts by oxidation the haematoxylin into haematin One gram of haematoxylin requires exactly 0 177 g. potassium permanganate.

With this stain the slide is stained for 10—60 minutes It is then differentiated in 0.2 % HCl until the blood drop is only a pale colour (a few seconds only) It is then washed in distilled water, placed in running water until a blue colour returns (alkalization), and finally allowed to dry The whole drop should be covered with immersion oil and examined under low power for the microfilaria When a microfilaria is found under low power, the oil immersion lens should be swung on, and the parasite can then be identified. In cases where the number of microfilariae in the peripheral blood is scanty, they may be concentrated from the venous blood.

To a quantity of venous blood is added several volumes of the following *Concentration method*

solution: 5% formalin, 95 cc.; acetic acid, 5 cc.; concentrated alcoholic gentian violet solution, 2 cc. The mixture is then centrifuged. The microfilariae, which are stained a blue violet, are found together with the leucocytes in the sediment.

Microfilariae of Onchocerca may be seen in wet preparations of tissue fluid in stained preparations as with the blood microfilaria.

Stool preparations

Stool examination for protozoa

Not all the protozoa that are pathogenic to man are capable of being stained well enough with Giemsa for purposes of identification. This applies especially to the majority of protozoa that inhabit the intestine, as well as to helminth ova and larvae.

Wet preparations

Examination of these is, initially, best made in wet preparations. For this purpose, a small portion of stool (or a mucous flake if they are present in the stool), is transferred to the slide with a wooden applicator or other suitable burnable or heat-sterilizable object (platinum loops are generally unsatisfactory, not being rigid enough). The stool specimen is mixed on the slide with a drop of normal saline or Ringer's solution, and the mixture is then covered with cover glass. The initial examination should be made with the low-power lens, and suspicious objects investigated further with the high power. Vegetative stages of protozoa are only seen in freshly passed warm stools. The addition of a 2% watery eosin solution enables the protozoa and cysts to be seen more easily; they stand out as colourless bodies against a stained background.

Iodine preparations

Positive staining — as opposed to the previously described "negative" staining — may be achieved by the use of iodine; here the organisms are stained against a colourless or pale background. A stronger Lugol's solution than normal is advisable in order to produce a clear nuclear staining. A satisfactory composition is: — add 4 g. iodine to 6 g. KI in 100 cc. distilled water. Craig and Faust prefer d'Antoni's iodine to Lugol's, the former staining more rapidly and the solution being much more stable than Lugol's. D'Antoni's solution is: — 1.5 g. of pulverised iodine added to 100 cc. of 1% KI. Stand four days, filter. The solution is now ready for use and should be stored in well stoppered bottles to prevent loss of iodine Well stoppered, it may be stored for long periods

without alteration of its characteristics The solution may also be used to preserve samples of stool sent by post or otherwise to far away laboratories For this purpose the faecal sample must be thoroughly mixed with the iodine solution, and the sample tube carefully stoppered

Permanent preparations

If a wet examination of stool is not possible, or if a permanent preparation is required, the preparation may be fixed wet on the slide or cover glass Generally the medium itself possesses ample fixing properties to glass (this applies to stool, intestinal contents, mucus, and blood and tissue fluids) to prevent detachment from the slide when the still wet preparation is placed in the fixative If this is doubtful, a little egg albumin or inactivated blood serum may be added

Preparation of permanent slides

The stool should be spread — not too thinly — on a slide which has been previously marked with a diamond, and then immersed whilst still moist in the fixative A staining jar is most suitable for this process, and the jar can be used for all the further stages of the process up to the mounting in Canada balsam or Caedox. Preparations on cover slips, generally recommended for fluid stools, and expediently made in fours, are fixed by dropping them, face down into a Petri dish of the fixative The cover-slips are then turned face upwards with a pair of forceps (ivory or horn with sublimate fixatives), and remain thus with changes of solution

Fixing and staining

Intestinal protozoa (amoebae, lambliae, trichomonads) are best stained with Heidenhain Fix the slides in sublimate — alcohol (1 part 96 % ethyl alcohol and 2 parts concentrated (7 %) aqueous mercuric chloride solution) to which before use a few drops of glacial acetic acid are added. For mordanting use a fairly fresh solution of 4 % aqueous iron alum, after use, this solution should be discarded, not poured back into the bottle Then stain with a 1 % alcoholic solution of haematoxylin made as follows dissolve 1 g. haematoxylin in 10 cc 96 % alcohol, then add 90 cc distilled water. Allow to ripen in air for several weeks, during which time the solution becomes quite dark. After use the solution may be used again if filtered. The staining process is as follows

Heidenhain's staining

Sublimate alcohol 20 minutes
Iodine alcohol (70 % alcohol to which tincture of iodine is added until light brown) 20 minutes
70 % Alcohol 30 minutes
4 % Iron alum solution 1 hour
Then give a short rinse in water

Haematoxylin 1 hour.

Again rinse rapidly in water.

Differentiate in 2% iron alum
(1 part of 4% iron alum mordant plus 1 part distilled water).

Using four slides differentiate them for 1, 2, 3 and 4 minutes each and then thoroughly wash in running water.

Carry over to 70%, 96% and then absolute alcohol (1 minute each) into xylene.

Mount in Caedax or Canada balsam under a cover slip.

Gönnert and Westphal have published a simpler procedure by means of which only one preparation is necessary for every stool. By means of a cover glass a long smear is made on a slide (this smear should not contain uneven masses, otherwise the cover glass will not lay properly, thus making oil immersion examination difficult). Immediately after the smear, the preparation is fixed in a staining jar in which many slides may be treated simultaneously. Over-fixing does not affect the staining The procedure commences as that given above for Heidenhain's method but diverges in the differentiation stage. Following the rinsing after haematoxylin staining, 30 cc. of 2% iron alum is poured into the staining jar and this is followed at one minute intervals by 20 cc., 20 cc., and finally 30 cc. of the iron alum. After a total of four minutes, all the solution is poured away and the slide is washed thoroughly with running water. The process is then continued as for the original Heidenhain's stain, i. e., through alcohols, etc. In this manner one slide contains a preparation which has been differentiated for four different periods of time, the different portions of the slide lying in parallel strips. Microscopic examination permits the most satisfactorily stained portion to be chosen With Heidenhain's stain, counter-staining is occasionally desired, and for this eosin, acid-fuchsin, or light green (in a 1% or weaker aqueous solution) are satisfactory. By this method the protoplasm is stained a different colour from the nucleus. The slide should only be dipped for a short time in a counter-stain before it is taken through alcohols into xylene, and embedded.

Craig and Faust's staining

Craig and Faust recommend in place of Heidenhain's stain, a rapid staining with iron haematoxylin: —

Fix in sublimate alcohol at 60° C. for two minutes.

Transfer to 70 % alcohol, iodine alcohol (70 % alcohol plus tincture of iodine until a port wine colour is reached), 70 % and finally 50 % alcohol 2 minutes each.

Then rinse in running water for 2 minutes

Immerse in 20 % aqueous iron alum at 40° C. for 2 minutes

Wash in running water 3 minutes

Stain in 0.5 % aqueous haematoxylin 2 minutes

Rinse in running water 2 minutes

Differentiate in cold iron alum solution The time required may be controlled microscopically

Wash in running water 10—15 minutes

Take slide through 70 %, 80 %, 90 % and absolute alcohol, 2 minutes each, into xylene, mount in Canada balsam or caedax

Mann's stain

A most useful method for amoebae is Mann's methyl blue — eosin staining in Dobell's modification. The stain is made as follows 35 cc of 1 % aqueous eosin solution plus 35—45 cc of 1 % aqueous methyl blue (not methylene blue) in 100 cc. of distilled water Stain 30 minutes or longer, wash in water and differentiate for one-half to five minutes in 70 % alcohol containing two to three drops of concentrated orange G per each 8 cc. of alcohol

Delafield's stain

Another staining method for wet-fixed preparations which is applicable to all protozoa and which was used for the staining of the microfilaria shown in plate XIX, is Delafield's haematoxylin stain, which is now briefly mentioned The stain may be purchased ready-made, or can be prepared in a laboratory. This can be done as follows· — 4 g haematoxylin are dissolved in 25 cc. of absolute alcohol, 52 g of ammonium alum are dissolved in 400 cc. of warm water Mix both solutions and allow the mixture to stand in an open flask (protected, however, from the entry of dust) for three to four days It is then filtered and 100 cc. glycerol plus 100 cc. methyl alcohol are added It is filtered again and the bottle is allowed to remain open until the solution attains a deep violet colour The ripe soluton is used in a dilution of 1 10 or 1→20 and, in order to avoid stain depositing on the slide, this dilution should be filtered before use. To obtain the best possible staining it is better to overstain for up to 24 hours, and then differentiate under the microscope with a solution containing 0.5 cc. of hydrochloric acid, 70 cc of 70 % alcohol and 30 cc. glycerol.

Stool examination for helminths Provoked stools are more productive of positive results than are normal stools. Complete adult helminths, tapeworm segments, larvae and ova of intestinal worms, flukes and the various schistosomes, as well as the intestinal protozoa can be found by this method.

Macroscopic examination For the detection of adult worms or their larvae, the stool is emulsified with water and strained through wire gauze or a hair sieve with the help of a jet of water. It may also be strained through two sieves, one on top of the other, the underneath sieve having a much finer mesh in order to hold back the finer particles. Further examination is best made by transferring the parasites into physiological saline, because any lengthy stay in water *tends to damage them. The microscopical demonstration of helminth* ova and larvae in wet preparations is carried out according to the technique for intestinal protozoa given on page 10. Hookworm larvae are conspicuous by their motility.

Concentration methods When direct examination of stool gives negative results the concentration method should be tried, for this is often positive when a direct smear is negative.

Sedimentation After allowing the stool to sediment, it is rubbed up with water and allowed to sediment again for 15—30 minutes. This should be done several times. The helminth ova and larvae sink to the bottom and the fine particles of stool which remain in the suspension are poured or siphoned off. This process may also be combined with the sieving method given above.

Centrifugation The stool may also be centrifuged by carefully mixing one part of stool with 10 parts of water, pouring through a gauze sieve into a centrifuge tube and centrifuging at moderate speed The supernatant is then poured off. Re-suspension and recentrifuging is carried out as given in the zinc sulphate technique on page 16.

Miracidia hatching test Schistosome ova may be demonstrated both in urine and stool sediments. If these ova are found in the deposit, the miracidia hatching test may be carried out If it is thought that schistosome ova are present, then the stool washing is best carried out with saline rather than water in order to avoid a premature hatching of the miracidia. When the supernatant fluid is finally clear, the deposit is suspended in cold tap water (less than 15° C.) and the centrifuge tube (or cylinder if the suspensions have been decanted) is placed overnight in the refrigerator. The following morning

the water is poured off and replaced with warm tap water at 30—40° C., and the vessel placed in the sun or under a bright electric lamp. Under the influence of light and warmth the miracidia hatch within a matter of minutes to hours. They may be seen with a magnifying glass as highly motile fine white threads (view against dark background) S. mansoni are seen mostly towards the bottom of the vessel, S. japonicum mostly towards the top.

NaCl concentration methods are especially suitable for nematode ova, and for certain tapeworm ova and protozoal cysts. The method depends upon the fact that these ova and cysts have a lower specific gravity than the stool particles, and in a saturated salt solution float to the surface — where they may be recovered in a reasonably clean state Mix thoroughly in a small beaker, tin, or small Erlenmeyer flask, 1 part stool with 20 parts of saturated NaCl solution (37.7 g NaCl → 100 cc. water) The first few drops of saline are added and the stool rubbed up into a paste; then more saline is added with continuous stirring until the whole is emulsified. After 15—45 minutes the ova may be removed from the surface of the liquid with a wire bent into a square loop of about 1 cm. This loop is placed, parallel, on the surface of the liquid and a loop of water removed onto a slide. Usually 3 drops are brought onto the slide, one next to the other, and the drops examined without covering The wire loop must be flamed after use to destroy any ova still attached.

Saline concentration method

Another method is to place a slide across the top of the beaker and then to fill the vessel until the underside of the slide meets the top of the liquid This is then left for 15—45 minutes when the ova floating to the surface will stick to the underside of the slide. This may then be inverted and examined Alternatively, cover slips may be placed on the surface of the liquid and then transferred to microscope slides. The slides or slips should not be left longer than 45 minutes as the ova begin to sink again after this period

Apart from saline, other substances may also be used for concentration methods These substances increase the specific gravity of the solution and among those commonly employed are zinc sulphate, sugar, glycerol and potassium hydroxide The simplest and best concentration method both for protozoal cysts and helminth ova and larvae is the zinc sul-

phate centrifugation technique of Faust and his co-workers. This is as follows

Zinc sulphate concentration method

A suspension of stool is prepared by rubbing up one part of stool and 10 parts of warm tap water: 10 cc. of this suspension are passed through a layer of damp gauze laying in a small funnel. The filtrate is collected in a centrifuge tube or a Wassermann tube and centrifuged for one minute at about 2,500 rpm. The supernatant is poured off, 2—3 cc. water are added to the sediment which is then shaken up or rubbed up in this water. When the sediment is again suspended, the tube is filled with water. Centrifugation and re-suspension are repeated until the supernatant fluid remains clear. When this stage is reached the supernatant fluid is poured off and 3—4 cc. of zinc sulphate solution (33% solution of a specific gravity of 1.18) are added, the sediment shaken up or stirred up, and more zinc sulphate solution is added until the tube is filled to about 1.5 cm from the top. The tube is then centrifuged for about one minute at high speed. With a platinum loop, one or two loops of the surface fluid are transferred to a clean microscope slide, one or two drops of d'Antoni's iodine added, and these are well mixed on the slide to obtain an even staining. This method is the most suitable for the simultaneous demonstration of protozoal and helminth ova or larvae in a natural undistorted state.

Telemann's method

A further concentration method which may be used for all forms of helminth ova found in stool is Telemann's method From a number of different places in the stool sample, small pieces of stool are placed in a test tube containing about 7 cc. of diluted hydrochloric acid (one part conc. HCl plus 1—2 parts water). The total mass of the stool material should be about the size of a bean. With a glass rod or small piece of wood, the stool is rubbed up against the inner side of the test tube and the stool mass is then well shaken to produce a fine suspension. An equal quantity of ether is now added and the suspension again well shaken. To remove the larger stool particles the mixture is then poured through a wire sieve or gauze lying in a small funnel. The suspension is then centrifuged for one minute with a hand centrifuge or it may be allowed to settle in a conical glass. After centrifugation or standing, the mixture consists of four layers as follows: a top, yellow, ether layer; a layer of detritus; an HCl layer; and a sediment in the bottom of the

vessel or tube which contains muscle fibres, vegetable cells and the helminth ova. After pouring off the three top layers — it may be necessary here to loosen the layers from the sides of the glass vessel with a wire or thin glass rod — the deposit should be broken up and a drop of this transferred to the microscope slide The drop is then covered with a microscope slide and examined. The sieve used should either be flamed if it is wire, or burnt if it is cloth.

General observations

In the previous sections dealing with the preparation of microscope specimens, those techniques have been chosen, the knowledge of which will be of use to the tropical physician in his practice. In the section that now follows, reference will repeatedly be made to this preceding section. As well in the special sections are given details of the microscopical demonstration of pathogens which are of interest only for the particular disease in question and for this reason they have been included within the special sections Both in the preceding section and in the following section the origins of the staining methods described are named. References to the literature, however, have been dispensed with because these are generally not available to the practitioner in tropical countries. The names may also be found in the standard textbooks of tropical medicine However, we shall be pleased to give information concerning the sources of the special literature upon request

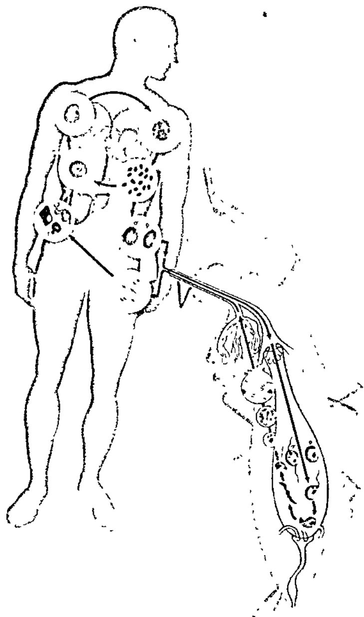
The Causative Organisms and their Microscopical Detection

Malaria

Malaria is to-day still the most important of the diseases which are transmitted by insects. The most striking symptom of the disease is the periodic fever. The interval between the attacks of fever has for two types of malaria designated the type in the older terminology. To-day the types are generally called after the causative plasmodium. In vivax malaria the fever occurs every third day and thus gave the name "tertian" to the disease. Plasmodium malariae produces a fever every fourth day and thus this type of malaria is referred to as "quartan". In falciparum malaria, also referred to as "sub-tertian" or "malignant tertian", the intervals between the fever are irregular. Frequently a new attack of fever occurs before the old one has disappeared. Another variety of tertian malaria is that caused by Plasmodium ovale. Although this type cannot be clinically differentiated from vivax malaria, the causative organism differs morphologically from Plasmodium vivax, as is later mentioned.

Malaria in General (Plate I)

The clinical symptoms of malaria are exclusively produced by those forms of the parasite which occur in the blood, i. e , which develop in the red blood corpuscles At the conclusion of their development they burst out from the red blood cell as merozoites which then enter new erythrocytes. This is apparent clinically, and demonstrable by the microscopical examination of the blood. This erythrocytic cycle, however, is only one part of the complete development of the malaria parasite which occurs in mosquito and man. Plate I shows this cycle of Plasmodium vivax. The sporozoites which are inoculated into the body by the bite of an infected [illegible] enter the [illegible] of the skin [illegible] salivary juice from the mosquito and, at first, enter the general [illegible]



lating blood. The injected sporozoites are taken up by body cells which, as far as we know, are chiefly the cells of the liver. In these cells the exoerythrocytic cycle, which at first is known as the pre-erythrocytic cycle, commences (2). In this phase parasites develop containing numerous nuclei. The length of this incubation period is about 10 to 14 days in both falciparum malaria and vivax malaria However, in vivax malaria it may extend to 200 to 300 days. In quartan malaria the incubation period is approximately 2 to 3 weeks (longer incubation periods have not been investigated in this form of malaria). After these respective incubation periods, the first merozoites are released into the blood stream and these then commence the erythrocytic (schizont) cycle In falciparum malaria, the length of the exoerythrocytic phase is no longer than the incubation period, and at the end of this incubation period the exoerythrocytic parasite forms enter the blood either all at once, or successively at brief intervals. They then institute the erythrocytic phase, i e, the generally seen microscopic phase. After the outbreak of a clinical attack of falciparum malaria it may be concluded that no more exoerythrocytic parasite forms are present in the body The development cycle in vivax and quartan malaria is different In these forms only a part of the exoerythrocytic parasites enter the blood at the end of the incubation period to form an erythrocytic cycle The remainder persists in the host's liver cells without producing clinical symptoms This reservoir gives rise to far more merozoites than those produced from the initial inoculation and development

After the erythrocytic cycle responsible for the clinical symptoms has finished, there may later arise from the persisting exoerythrocytic parate reservoir a new batch of merozoites which produces another infec...on of the blood and thus a malaria relapse This relapse may recur repeatedly The total number of exoerythrocytic parasite forms may also in vivax malaria be exhausted after the first malaria attack as in falciparum malaria, or after the first relapse This depends upon the number of injected sporozoites and, or the number of infected biting mosquitoes It is not certain, therefore, that a vivax malaria infection will lead to several clinical attacks of malaria On the other hand the number of relapses, i e., the influx of merozoites from their reservoir into the blood may number twelve or more The period of time during

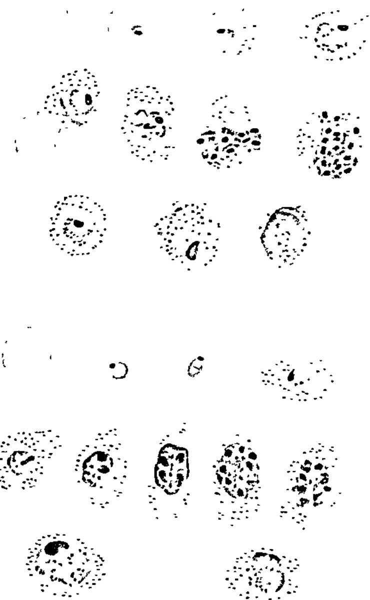
which, in vivax malaria, the reservoir of existing exoerythrocytic forms is finally exhausted, is generally not greater than two years. Only in quartan malaria are relapses seen many years after the original infec- [illegible]

[illegible] present, probably also the same as that of vivax malaria. Plate I, Nos 3, 4, 5, 6 and so on, give a diagrammatic representation of the erythrocytic cycle, i.e., the growth of the parasites in the red blood cells, the release and repenetration of the merozoites. The disruption of the red blood cells and the simultaneous release of the merozoites occasion the commencement of a fever attack. The Plate shows that not only schizonts develop from the merozoites but also gametocytes. These are the male and female sexual forms (6—7) These forms may be taken up by the feeding mosquito and thus begin the sexual cycle. This cycle, known as sporogony, concludes with the migration of the mature sporozoites into the salivary glands of the mosquito. Plate I also shows this development occurring (8—16).

For the microscopical diagnosis of malaria only those parasites are concerned which, as has been described above, are present in human blood. These are primarily the schizonts which are alone responsible for the clinical symptoms, and secondly the gametocytes. The search [illegible] forms in the liver is only possible in liver [illegible] interest. The [illegible] asexual forms, have definite characteristics for each form of malaria. These forms are now described.

Vivax malaria (Plate II, top half)

The top half of Plate II shows Plasmodium vivax parasites as they are seen on a blood slide stained with Giemsa stain (see page 5). They appear first as small ring forms in the erythrocytes, they then develop until they are [illegible] a normal red blood corpuscle. The infected blood cells may thus themselves increase in size until they are one-and-a-half to twice the normal size and appear "blown-up". The marked increase in size of the infected erythrocytes is a characteristic of vivax malaria. The parasites frequently show, when well stained, delicate red dots, known as Schüffner's dots. As the parasites grow, these dots



increase. This is also a characteristic of vivax malaria. As the ring develops, fine yellow-brown pigment granules — breakdown products of haemoglobin — develop. Generally, the ring forms are no longer seen 24 hours after a fever attack. The original single nucleus has in this time divided into 12 to 24 parts. The final form consists of a mulberry arrangement (morula). The average number of nuclei in a morula is characteristic for the type of malaria and, therefore, important for differential diagnosis. After the disruption of a morula the young, round or oval, individual merozoites penetrate new erythrocytes and develop again into ring forms. This development in vivax malaria takes 48 hours and, therefore, the merozoites are released every third day to produce a new fever attack. Also recognised is the form of vivax malaria known as duplicate tertian, in which two populations of erythrocyte parasite forms exist. The development of these two populations is staggered so that a fever attack in this form of malaria occurs every day.

Plate IV (top half) shows the thick drop blood preparation, Giemsa stained (see page 7), demonstrating vivax forms of malaria. This shows that when correctly made, the red blood corpuscles are almost completely laked. In the ghost erythrocytes, distributed among the other blood elements (leucocytes, etc.), the malaria parasites can be observed in different developmental stages as delicate rings, as trophozoites and as mature morula forms with definite pigment deposits, gametocytes may also be seen in this preparation

Vivax malaria (Plate IV top half)

Plate III (top half) shows the parasites of Plasmodium falciparum as they appear in a thin blood film when stained with Giemsa stain. Only the erythrocytic forms are seen here. Falciparum rings are smaller and more delicate than the other forms, and it is common to find several rings within one erythrocyte. The nucleus is stained a vivid red and is often present as a double dot. Even when the malaria is well developed the signet ring forms are almost always the only forms found in the peripheral circulation. Although the gametocytes may not be seen in the blood film, a diagnosis of falciparum malaria can be made when only ring forms are found in the blood The presence of schizonts in the blood constitutes a grave symptom in falciparum malaria. Generally the division of the parasite occurs in the capillaries of the inner organs (such as the brain and kidney). In these organs one sees the very small

Falciparum malaria (Plate III top half)

schizonts which do not completely fill the erythrocytes. The morula disintegrates into 8 to 24 merozoites, *and the developmental cycle takes* between 24 to 48 hours.

The early stages of gametocyte development in falciparum malaria are similar to those of vivax malaria for both have discs without vacuoles They extend to an oval shape and dilate the red blood corpuscle in which they are contained. Later development to the crescent forms is characteristic of falciparum malaria, not being seen in any other type The protoplasm of the female crescent stains a definite blue with a compact, small, usually central, nucleus which stains red (Giemsa). The male crescents are frequently smaller, paler, and more pink. Their red chromatin nucleus, which is frequently po[illegible] to be [illegible]buted ar[illegible] the whole gametocyte a pale [illegible]. In the peripheral circulation [illegible] sexual forms are seen, and falciparum gametocytes indicate that the malaria is of long duration.

The red blood corpuscles do not increase in size when parasitized by falciparum ring forms Intensive Giemsa staining shows the characteristic Maurer's dots, as red flecks or small spherical bodies.

Falciparum malaria (Plate IV lower half) The lower half of Plate IV shows falciparum malaria as characteristically seen in thick drop preparations stained with Giemsa. This preparation has apparently been taken some time after the clinical outbreak of the fever because in addition to the numerous ring forms there are also present male and female gametocytes. The ring forms are most noticeable in this preparation by the vivid staining of the chromatin dots.

Quartan malaria (Plate III lower half) The lower half of Plate III shows the different developmental stages of the erythrocyte phase of Plasmodium malariae *in a thin blood film* stained with Giemsa. These forms are not seen in great numbers in the peripheral blood, and for this reason it is advisable to look for them at the end, or at the edges, of the blood smear. The quartan rings are, at *first, just as small as the tertian, but as they develop they extend into* narrow quartan bands across the erythrocyte. In this band one notices the bright red chromatin nucleus as well as the yellow brown pigment granules which are frequently collected at the edge of the band. With maturity the band grows broader and eventually completely fills the red blood corpuscle. The number of merozoites is generally 8 to 12,



frequently symmetrically placed about the centrally deposited pigment, to produce the "daisy forms". The asexual developmental cycle of the parasite takes 72 hours and, therefore, an attack of fever is to be anticipated every fourth day. The quartan gametocytes resemble those of the tertian, but usually completely fill the red blood cell The red blood corpuscle parasitized with a quartan parasite is not enlarged and pale as is the case with Plasmodium vivax infections

Plasmodium ovale (Plate II lower half)

Plasmodium ovale is to-day accepted by most authorities as a definite species. The clinical symptoms resemble those of vivax malaria In their form and size, however, the parasites resemble those of quartan malaria without having the band form which is characteristic of the quartan species The young trophozoite is relatively small but as this grows it expands the red blood corpuscle which is frequently then oval in shape It is this characteristic that gives the name to the species. The parasitized red blood cell does not enlarge and become pale, but has, similar to vivax malaria, a marked Schuffner's stippling and an irregular fringed edge. The schizonts of Plasmodium ovale contain fewer merozoites than those of Plasmodium vivax. The developmental cycle takes 48 hours

Treatment

As is stated in the introduction, all clinical symptoms of malaria are caused exclusively by the schizonts of the erythrocytic phase The most important chemotherapeutic for the treatment of an attack of malaria is, therefore, an effective schizonticide As such, Resochin (see page 68) has been internationally approved In falciparum malaria after the first attack, no exoerythrocytic parasite forms persist and, therefore, a thorough treatment with a schizonticide radically cures this type of malaria without any fear of a relapse occurring In vivax, ovale, and quartan malaria (after the erythrocytic schizonts which are responsible for the clinical symptoms have been destroyed) there are still to be reckoned with the persistent exoerythrocytic parasite forms which cause the relapses. If these relapses are to be avoided, then at the conclusion of a Resochin course either Primaquine or Plasmoquine (see page 67) should be administered.

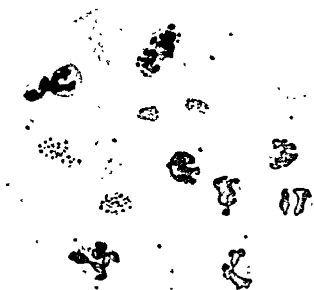
Prophylaxis

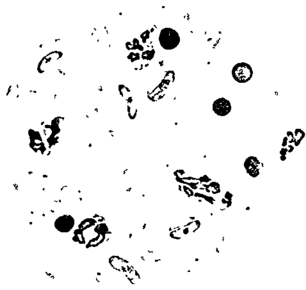
A causal prophylactic which destroys the inoculated sporozoites before they can develop into pre-erythrocytic or exoerythrocytic forms, is, at the present time, unknown. The most practical method of prophylaxis available at the moment is suppressive treatment (chemoprophylaxis)

For suppressive treatment a drug is necessary which will destroy the schizonts growing from either the exo- or erythrocytic parasites immediately they are formed, and should be capable of achieving this when regular small doses are administered, thus preventing the appearance of any clinical symptoms. In this early stage, the number of schizonts is practically always sub-microscopical so that they are not demonstrable in the blood. Here, also, Resochin in weekly doses has proved perfectly reliable. The method of terminating a suppressive treatment depends upon the conditions of infection. If the infection is exclusively that of falciparum malaria, the suppressive treatment may be given for two weeks and then concluded with a single therapeutic dose. All the schizonts will then have been destroyed, and the exoerythrocytic parasites can no longer exist. In contra-distinction to falciparum malaria, vivax and probably also quartan malaria present the possibility of a late relapse if the persistent exoerythrocytic parasites are present in the body. These parasites may enter the general circulation as merozoites, develop, and cause an attack of fever if no schizonticide is present in the blood to interrupt the development. If the danger of this late relapse is to be avoided Resochin should be continued for two weeks after leaving the malarial area and, at the same time, a daily dose of Primaquine (see page 68) should be taken.
Blackwater fever, producing the characteristic acute haemolysis, is today, following the introduction of the synthetic schizonticides, practically unknown. However, especially by repeated attacks of malaria, or when concomitant parasites exist in the body (helminths etc) anaemias may appear. In this case, a rapid regeneration of the blood picture may be achieved with the liver extracts such as Campolon and Campoferron (see page 60).

African Trypanosomiasis

The most important symptoms of African trypanomiasis (Sleeping Sickness) are irregular fever, lymphadenitis, transient local oedema and erythema, psychological and nervous disturbances, increasing cachexia and, in the terminal stages, an overwhelming desire to sleep.
The transmission of the disease from man to man is effected by a bite





of the tsetse fly of the genus Glossina. This, by sucking the blood of an infected case, takes up the trypanosomes. Twenty to 34 days after this, the glossina fly becomes infective for other men. In this period the parasites undergo a reproductive cycle in the fly before they enter the salivary glands of the insect preparatory to the inoculation into a new host. The incubation period of the disease is about 2 to 3 weeks. Between 2 to 5 days after the bite of the infected glossina fly there occurs at the site of the bite an inflammatory swelling which is known as the trypanosome chancre. This is the localised primary effect.

Causative organisms (Plate V and VI upper half)

The causative organisms of African trypanosomiasis, Trypanosoma gambiense and Trypanosoma rhodesiense, are flagellates of fishlike form. They are approximately 2 to 3 times as long as the red blood corpuscle. When stained with Giemsa (see page 5) the nucleus which lies in the middle of the flagellate is visible. A second structure which is demonstrable with nuclear stains, is the small blepharoplast which is seen towards the posterior of the parasite From this blepharoplast there extends a flagellum which is a threadlike process serving for the propulsion of the parasite. This undulating flagellum continues past the posterior of the parasite. The protoplasm of the trypanosome stains blue, the nucleus, blepharoplast and flagellum stain red. In the protoplasm are also seen the volutin granules which stain dark red to black.

Diagnosis

For the diagnosis of trypanosomiasis it is always desirable to demonstrate the parasite when this is possible. The earliest time at which the parasite may be seen is at the initial chancre This should be scarified with a scalpel after the area has been made hyperaemic by pinching between the fingers. A few drops of the tissue fluid issuing from the scarification should be examined for the trypanosomes These are usually conspicuous by their lively movements The trypanosomes may also be demonstrated by puncture of the superficial lymph glands, especially those in the neck. Lymph gland puncture should be made with a needle of medium size — if a needle is too thin it may block. Ordinary water is lethal to trypanosomes, and before lymph gland puncture special attention must be paid to seeing that the interior of the hypodermic needle is quite dry. After puncture one drop of the lymph is placed upon a microscope slide, covered with a cover slip and examined with a 4 mm. objective with the iris of the condenser partly closed

The trypanosomes may also be demonstrated from the sixth to the twelfth day after infection in a thin blood smear or thick blood drop These are best made during the fever. They should be stained with 1:10 Giemsa for about 20 minutes (see pages 5—7) and then examined with an oil immersion lens. In T. rhodesiense infection, the diagnosis is easier because more parasites are present. The trypanosomes may also be concentrated by the following process: in a 10 cc. syringe, 1 cc. of a 6% sodium citrate solution in a 0.9% sodium chloride solution is drawn up and the syringe is then filled with blood from a vein. This mixture is centrifuged (a hand centrifuge for 10 min.) Underneath the erythrocyte layer will be found a grey thin layer of leucocytes; with a pipette this leucocyte layer is removed and one or two drops placed on a slide and examined as above.

In the secondary stage of trypanosomiasis, trypanosomes may also be seen in the cerebrospinal fluid. In this stage if no parasites are found in the CSF, a definite increase in the cell count should make one suspicious.

Treatment

When the cerebrospinal fluid is negative, Bayer 205 (see page 58) is sufficient for therapy. When the CSF shows an increased cell count or when trypanosomes may be demonstrated, Bayer 205 should be combined with tryparsamide.

Chagas' Disease

Another acute infection with trypanosomes is Chagas' disease which is limited to South and Central America. The disease is accompanied by fever, diarrhoea and lymphadenitis and, in the chronic stages, a characteristic myocarditis occurs.

The insect host which transmits the disease is a large bug of the genus Triatoma. The natural reservoirs are the armadillos, marsupials, cats, and dogs.

Causative organism (Plate VI lower half)

Trypanosoma cruzi, the causative organism is smaller than Trypanosoma gambiense. Especially noticeable is the large size of the blepharoplast of Trypanosoma cruzi. The parasites are not plentiful in the blood. After penetrating the cells of the RES, heart or skeletal muscle, the trypanosomes lose their flagella and assume the form of leishmania



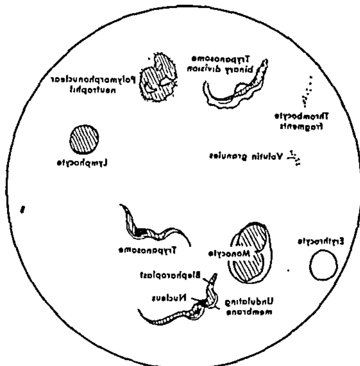




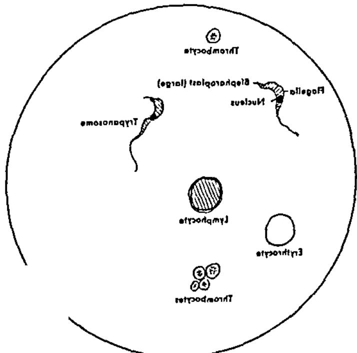






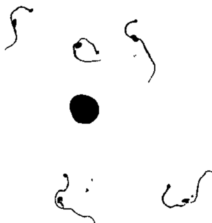


Trypanosoma rhodesiense, Blood smear
Giemsa stain, magn. × 1100



Trypanosoma cruzi, Blood smear
Giemsa stain, magn. × 1100





Only in this form does Trypanosoma cruzi reproduce by mitotic divisio such division was once thought to be schizogony (hence the old nar of Schizotrypanum)

Clusters of parasites develop intracellularly, these clusters conta hundreds of parasites in a characteristic grouping. The leishmania-li parasites are, before entry into the circulation, transformed into t flagellate forms. This change between the blood and tissue phase of t parasite is continually repeated

Diagnosis of the disease depends upon the identification of the parasi In this connection only the blood form is capable of being demo strated in practice, the tissue forms being only obtainable with di culty by means of organ biopsy Because of the small number of pa sites present in the blood, microscopic diagnosis is difficult (see pag 5 and 7 for Giemsa staining of thin films and thick drop) A conce tration method by centrifuging (as for sleeping sickness) is possib When the blood is negative, animal inoculation will often help (mi guinea pigs and young dogs are suitable animals) Another method to feed a parasite-free bug of the Triatoma genus on a patient suspect of the disease After a certain interval the intestinal tract of the insec examined for the developmental stages of Trypanosoma cruzi

Leishmaniasis

(a) Kala-Azar

(Visceral leishmaniasis, tropical splenomegaly)

Visceral leishmaniasis is a progressive general infection of ma months duration, accompanied by irregular fever and marked enlarg ment of spleen and liver, leading gradually to severe anaemia ar when untreated, death.

Leishmania are conveyed by sandflies of the phlebotomus family.

The incubation period may be less than 10 days, but occasionally more than 18 months

Leishmania donovani is a parasite which belongs to the trypanoso family. In the extended motile form with flagella, it is only seen in c ture In the human body (spleen, liver, lymph-glands, bone-marrow), t leishmania are found practically only intracellularly, and are non-mot

round or oval bodies about one-third the size of a red blood corpuscle. These contain a spherical nucleus and, in addition, a spherical or short rod-like blepharoplast. Leishmania in this form are generally found *intracellularly in large monocytes, but* in smears on slides they are sometimes found extracellularly. When stained with Giemsa, the protoplasm appears blue, and the spherical nucleus stains an intense reddish violet.

Diagnosis Diagnosis is confirmed by the finding of the causative leishmania. This is, however, not always an easy matter, because the parasites are present in the blood only in limited numbers. They are found here mainly in monocytes and in other leucocytes or macrophages. It is, therefore, advisable to examine the end of the blood smear where the leucocytes are usually present in quantity. The thick drop technique is not so suitable. Spleen puncture affords material rich in parasites, but because of *the dangers involved in this process it is usually avoided when possible.* Parasites are not so easily demonstrated in the liver and sternal punctures as in the spleen. The blood picture may also give a valuable confirmation of the diagnosis of Kala-Azar. In a well developed spleen tumour with chronic fever, a leucopenia and relative mononucleosis is suggestive of Kala-Azar.

Treatment For treatment, the pentavalent antimony preparations are generally preferred. Among these preparations are Neostibosan (see page 66) and Solustibosan (see page 71).

(b) Cutaneous and mucous membrane leishmaniasis
(Oriental boil)

Cutaneous leishmaniasis is a local infection which generally commences on an uncovered body area. At first a small papule is noticed which soon scabs over and then enlarges into a painless ulcer. This may after *several months resolve and heal with the formation of scar tissue.* The incubation period is, as with Kala-Azar, between two weeks and 18 months.

Causative organism The causative organism, Leishmania tropica, which is transmitted by phlebotomus insects, cannot be *differentiated from* Leishmania donovani. Another variant is Leishmania braziliensis, the causative organism of South American cutaneous and mucosal leishmaniasis.





Diagnosis is confirmed by the demonstration of the causative organism. Material for the examination is obtained by scarification of the edge of the ulcer with a scalpel. A drop of serum from the edge of a papule is also suitable The preparation is stained with Giemsa stain (see page 5). Diagnosis

Treatment is by the local injection of the foci with Solustibosan (see page 71) or with Atebrin solution (see page 56) This injection may be given around or under the focus. Ulcerated boils should be covered with collodion in order to prevent the exudation of fluid. General treatment is with Neostibosan (see page 66), Solustibosan and, especially in South American mucosal leishmaniasis, with Fouadin (see page 62) Treatment

Amoebiasis

Amoebiasis in its intestinal stages causes the development of ulcers in the large intestine and more or less marked dysenteric symptoms The extra-intestinal complications are caused by the migration of the parasites through the wall of the intestine into the liver, lung and other organs The disease is transmitted by the resistant cysts which are present in impure water or in unclean foods The cysts may also be transmitted by flies if these have the opportunity to visit human faeces and then land on food The acute symptoms may develop two to four weeks after the infection or they may, after a much longer latent stage, suddenly erupt when certain conditions enable them to do so.

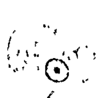
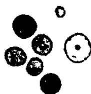
Entamoeba histolytica in their vegetative stage exist in two forms which may be differentiated morphologically by their size, and physiologically by their location and mode of living The small forms known as minuta forms live in the lumen of the intestine where they reproduce by binary division They are not responsible for any special clinical symptoms In certain circumstances, these minuta forms may penetrate the wall of the intestine and there develop into the large race. These magna forms then multiply in the tissue; it is the magna forms alone which are responsible for the destruction of tissue, and for the production of the clinical symptoms The cysts, i.e, the forms which are responsible for the transmission are only produced from the minuta forms These cysts are also non-pathogenic as far as the carrier is concerned. Causative organism (Plates VIII and IX)

Diagnosis In acute amoebic dysentery the magna forms may be found in the stool (especially in the flakes of mucus) These should be transferred to a microscope slide, mixed with one drop of physiological saline, covered with a cover slip, and examined. In chronic amoebic dysentery, generally only the minuta forms or the cysts appear in the stool: the magna forms only rarely. The minuta forms show a vacuole whose contents are homogenous for this form obtains its nourishment primarily from dissolved substances Occasionally, however, bacteria also are taken up In the magna form one finds the protoplasm as a compact mass in which erythrocytes are often present. The demonstration of these phagocytosed erythrocytes in the protoplasm of the magna form is of great importance diagnostically. When staining with Heidenhain's stain (see page 11), small chromatin bodies are seen on the membrane of the nucleus of the amoebae In the interior of the nucleus are small granules which are, in the minuta form either centrally placed or on the edge, and in the magna form, generally central. The cysts are surrounded by a thin shell. The cysts at first contain only one nucleus but, later by binary division, produce four nuclei. These nuclei correspond to the nuclei of the vegetative form. The cysts also contain a glycogen vacuole which may be stained brown with Lugol's iodine. This vacuole is most noticeable in the mononucleate cysts, and in the course of time, as the cyst matures, the vacuole diminishes in size. When a mature cyst is swallowed, the gastric juice enables the four-nucleate amoebae to slip out. Later development produces eight mononucleate amoebae which become minuta forms These, by binary division, may continue to build cysts, or develop into magna forms as has been previously described.

Treatment For the treatment of all forms of amoebiasis, i. e , both the acute and chronic amoebic dysentery, as well as the extra-intestinal symptoms, Resotren is indicated (see page 70). Resochin (page 69) may be administered orally and parenterally only for the treatment of the extra-intestinal complications Yatren (page 73) is only used for the treatment of the intestinal symptoms.

Giardiasis

An infection with Giardia intestinalis can lead to the following symptoms. a feeling of heaviness in the epigastric region, anorexia, pains




Diagnosis In acute amoebic dysentery the magna forms may be found in the stool (especially in the flakes of mucus). These should be transferred to a microscope slide, mixed with one drop of physiological saline, covered with a cover slip, and examined. In chronic amoebic dysentery, generally only the minuta forms or the cysts appear in the stool: the magna form only rarely. The minuta forms show a vacuole whose contents are homogeneous. This form obtains its nourishment primarily from dissolved substances. Occasionally, however, bacteria also are taken up. In the magna form one finds the protoplasm as a compact mass in which erythrocytes are often present. The demonstration of these phagocytosed erythrocytes in the protoplasm of the magna form is of great importance diagnostically. When staining with Heidenhain's stain (see page 11), small bodies are seen on the membrane of the nucleus of the amoeba. In the interior of the nucleus are small granules which are, in the magna form, either centrally placed or on the edge, and in the minuta form, generally central. The cysts are surrounded by a thin shell. The cysts at first contain only one nucleus but, later by binary division produce four nuclei. These nuclei correspond to the nuclei of the vegetative form. The cysts also contain a glycogen vacuole which may be stained brown with Lugol's iodine. This vacuole is most noticeable *in the mononucleate cysts, and in the course of time, as the cyst matures,* the vacuole diminishes in size. When a mature cyst is swallowed, its destruction enables the four-nucleate amoebae to slip out. Later development produces eight mononucleate amoebae which become minuta forms. These, by binary division, may continue to build cysts, or magna forms, as has been previously described.

Therapy For the treatment of all forms of amoebiasis, i.e., both the acute and chronic amoebic dysentery, as well as the extra-intestinal symptoms, Resotren is indicated (see page 70). Resochin (page 69) may be administered orally and parenterally for the treatment of the extra-intestinal complications. Yatren (page 73) is only used for the treatment of the intestinal symptoms.

Giardiasis

An infection with Giardia intestinalis can lead to the following symptoms: a feeling of heaviness in the epigastric region, anorexia, pains











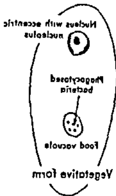




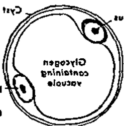




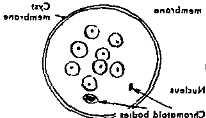


Vegetative form

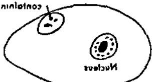


Binucleate cyst

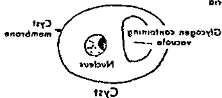


8-nucleate cyst

Entamoeba coli



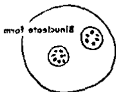
Vegetative form



Cyst

Iodamoeba bütschlii





Vegetative forms

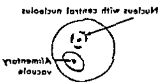
Dientamoeba fragilis



Vegetative form


4-nucleate cyst

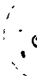
Endolimax nana



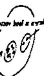
Entamoeba histolytica
(Trophozoite)
Small race, E. hartmanni

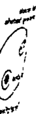
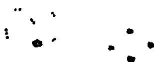
Note the characteristic nuclear structure of the different amoebae parasitic to man.
Heidenhain's haemotoxylin
Magn × 2000








resembling chronic cholecystitis, and relapsing diarrhoea of a spruelike nature. Hypacidity and intestinal disorders resulting from other disturbances must be taken into account as additional factors. The transmission of the disease is, as in amoebiasis, by means of the cysts.

Causative organism (Plate X)

Giardia (lamblia) intestinalis is a flagellate, somewhat resembling a split pear, which inhabits principally the upper section of the small intestine. The parasite forms cysts which are excreted with the stool. The vegetative form with its characteristic shape forms two symmetrical halves. Four pairs of flagella are attached to blephoroplasts. The cytoplasm also contains a characteristic para-basal body. The anterior half has a sucking disc by which it attaches itself to the intestinal membrane. The oval cysts are surrounded by a thick wall.

Diagnosis

In the stool the vegetative forms are generally only seen in diarrhoea. Otherwise these motile vegetative forms can rarely be found except when duodenal fluid is examined. The cysts, however, may commonly be seen in formed stools.

Treatment

Acranil (page 55) or Atebrin (page 56)

Spirochaetes

Yaws and Syphilis

Yaws (framboesia) and syphilis are caused by related spirochaetes which are transmitted from man to man either by extra-genital or genital contact. The diseases commence with a characteristic primary chancre or sore in the skin or mucous membrane. The secondary stage leads to a widespread roseola or pustular rash due to the systemic spread of the infection. The tertiary stage produces severe alterations in the skin and bones and, in syphilis, in the central nervous system as well.

Causative organism

The causative organisms of yaws and syphilis (Treponema pertenue and pallida) appear as delicate spirals with narrow corkscrew coils. It is not possible to differentiate these parasites either morphologically or by staining processes

Diagnosis

In yaws the parasites may be demonstrated either in the primary chancre or in the secondary pustules following light scarification and examination of the resultant exudate. In late granulating sores, an initial cleansing of the skin is necessary before examination to remove the con-

taminant flora. In the late florid lesions and the tertiary stages it is generally not possible to demonstrate the spirochaetes.

In syphilis, to demonstrate the spirochaetes which are generally only present to a limited extent, the initial chancre or the eroding secondary pustule should first be vigorously cleaned with cotton wool. This removes the secondary organisms and also stimulates the production of serum. Then, if the sore or chancre has already been treated with antiseptics, these must carefully be [illegible] and the chancre treated with saline compresses for a few days. It is necessary to obtain for the examination, tissue fluid which is as [illegible] as possible from the deep layers of the skin.

Dark Field

The best and easiest method of demonstrating spirochaetes is the examination by means of the dark-field condenser. The slide is prepared as follows: a cover slip held with forceps is brought on a drop of the issuing chancre serum. The cover slip is then placed on the microscope slide face downwards. Previously one drop of physiological saline solution should have been placed on this slide. The diluted serum then spreads out between the cover slip and slide. Upon examination the spirochaetes are seen as light bodies against a dark background. They possess a characteristic movement which is the rotation along their long axis and a flexion of the whole body.

Indian ink preparation

If the dark field examination is not possible then examination may be made by the [illegible] which depends on the negative [illegible] of the parasit[illegible]

The remainder of the parasites in the smear are covered by the stain. In [illegible] method a drop of Indian ink is placed at the end of a microscope slide and well mixed with a large drop of the serum under examination [illegible] thin blood [illegible] [illegible] mmers [illegible] background [illegible]

Staining method

[illegible] ink staining method [illegible] over a large part of the spirochaetes, it is possible to obtain better results by means of a positive staining method. For this, Giemsa long staining (page 8) and the following rapid staining method are suitable.





Tropical Ulcer

A tropical ulcer is nearly always found on the lower third of the lower limb. It commences with a blister which fills with a sero-sanguineous fluid After the bursting of this blister, the subcutaneous tissues are attacked and the process sometimes reaches the periosteum. The sloughing produces a gangrenous, fetid, crater-like ulcer.

Causative organism (Plate XI, lower half) In tropical ulcer at the edge of the ulcer is to be found a symbiosis of relatively thick spirochaetes of unequal length and spindle-shaped bacilli as in Vincent's angina.

Diagnosis The surface of the ulcer should be cleaned with cotton wool in order to remove the heavy bacterial flora that is always present. After cleaning, a little tissue fluid is taken from the depth of the ulcer or from the undermined edge The spirochaetes are usually present in considerable numbers matted together. It is generally necessary to dilute the serum with physiological saline in order to make the spirochaetes in these masses motile and microscopically definable. A film is made of the diluted ulcer fluid and this may be stained with a 1 → 10 solution of carbol fuchsin (see page 37). The negative staining process with Indian ink (see page 32) may also be used. Giemsa staining produces excellent pictures.

Treatment Pre-operational, and in support of surgical measures, Supronal orally or parenterally, or Badional may be given (see pages 57 and 71) For local treatment Marfanil-Prontalbin powder (see page 62) or Badional powder (see page 58).

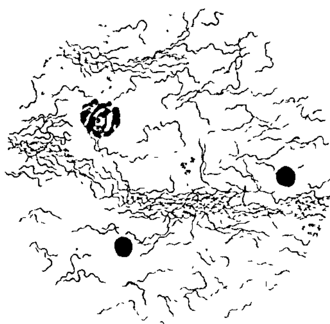
Cholera

The characteristic symptom of cholera is the rice-water stool which is passed almost continuously. This produces a water and mineral salt loss which, together with bacterial toxins, exert their detrimental influence on renal function and blood circulation and affect the course of the disease

The infection results from the ingestion of infected material.

Incubation period 1—2 days

Causative organism (Plate XII, upper half) Cholera vibrios are comma-like, slightly curved motile rods with a long terminal flagellum. These flagella are only visible when stained by special methods.





For examination one chooses a small mucous flake from the suspected stool and makes a smear of this on a microscope slide. The smear is heat-fixed and stained with carbol fuchsin solution (see page 37) diluted 1→10 with water. The stained preparation is then examined with an oil immersion lens. If, in the stool, other comma-like bacilli are present, then a microscopical diagnosis should be confirmed by culture and the specific agglutination reactions.

Diagn

A trial with Periston N · is indicated (see page 67).

Treatment

Plague

Plague occurs in man in the bubonic, pneumonic, septicaemic, and meningeal forms. These forms may pass one into the other. Plague is highly infectious, presents severe symptoms, runs a rapid course, and has a high mortality rate. The causative organism, Pasteurella pestis, is seen both enzootically and epizootically in rodents and, occasionally — with the flea as the intermediate host — affects man. In pneumonic plague, transmission by droplet infection is also possible. In the advanced stages, especially in bubonic plague, bacilli are usually present in common with staphylococci and streptococci. The incubation period of bubonic plague is 2—5 days and pneumonic plague 1—3 days.

Pasteurella pestis is seen as a non-motile, short, bi-polar staining, gram-negative, oval bacillus.

Causative organism (Plate XII, lower half)

For diagnosis lymph fluid should be obtained from an enlarged lymph gland by puncture, or the secretion from a plague bubo may be examined. In pneumonic plague the organisms may be found in great quantity in the watery, blood-tinged sputum. In septicaemic plague the bacteria may be found in the peripheral blood.

Diagnosis

For differential diagnosis, bi-polar staining should be employed. The smear should be dried in air and fixed with absolute alcohol for half a minute. The alcohol is then poured off. That alcohol remaining on the slide is then burnt off. Dilute methylene blue may be used as the stain, and it should be allowed to act for 2—5 minutes, or alkaline methylene blue (100 parts of 0.1 % potassium hydroxide plus 30 parts of conc. alcoholic methylene blue. This methylene blue consists of 20 g methylene blue in 300 cc. of 96 % alcohol) may be used. After staining, the slide

Polar staining

should be washed and dried. Bi-polar staining is also possible using Giemsa (see page 5). It should be remembered that other members of the Pasteurella group also stain bi-polarly.

Treatment Supronal and Solu-Supronal (see page 71) and Badional (see page 57)

Tuberculosis

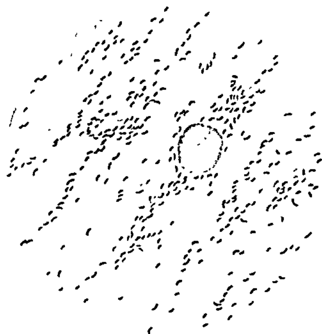
Tuberculosis is a chronic infectious disease which begins with a primary localised process in the lungs, manifesting itself as an organic disease, usually of the lungs. Transmission is by inhalation, ingestion in the food, or by inoculation of the bacillus.

Causative organism (Plate XII, [illegible]) *Mycobacterium tuberculosis*, human or bovine, is a delicate, frequently slightly curved, non-motile, non-sporing rod, which sometimes shows a knob-like end

Diagnosis Most important microscopic diagnosis is the demonstration of the bacilli in the sputum. They are seen here (as also in urine) mostly as single rods, but occasionally in small round, or elongated clumps.

For the examination of sputum, a small fragment of pus should be removed with forceps, and pressed out between two microscope slides. These slides, upon pulling apart, should leave an even thin smear. This is dried in the air, and fixed by waving gently two or three times through a flame. When bacteria are present only in small numbers, a concentration method should be employed. Equal parts of sodium hypochlorite and sodium hydroxide are mixed. Two parts of this mixture, and one part of sputum are mixed, well shaken and allowed to stand for 10—30 minutes. The homogenous sputum is then diluted to twice or thrice its volume with distilled water and centrifuged for 15—30 minutes at at least 2,000 r. p. m. The supernatant fluid is poured off and the tube left upside down for 10—15 minutes. The deposit is removed from the bottom of the tube with a platinum loop and then spread on a microscope slide with the help of a little of the non-concentrated sputum. The slide is dried carefully high over a flame, fixed and stained, and carefully washed. It is then dried (not with filter paper) in a warm current of air.

Examination of urine should always, in order to avoid confusion with smegma and other acid-fast bacteria, be made with urine obtained by sterile catheterisation. In urogenital tuberculosis the bacteria are easily





found in the centrifuged purulent deposit of the frequently bloody, cloudy urine. The sediment does not always adhere well to the microscope slide and it is advisable to add a trace of dilute egg albumin to the deposit

The most generally used stain is that of Ziehl-Neelsen The thin film is fixed with heat (the slide is waved three times through a Bunsen flame with the film side of the slide upwards) It is stained with hot carbol fuchsin solution for 2 minutes (carbol fuchsin solution should be heated until steam appears It should not be allowed to boil) The stain is then poured off; it is decolourised with hydrochloric acid until quite colourless, quickly washed in distilled water, counterstained with dilute methylene blue for 5—10 seconds, washed with distilled water and dried. Tubercle bacilli and other acid-fast bacilli appear red, the background, mucous, cells and other bacteria should only be stained light blue. Ziehl Neelsen stain

For this staining one needs

1. Carbol fuchsin solution 10 cc. of saturated alcoholic fuchsin solution plus 90 cc 5% aqueous solution of phenol
2 Hydrochloric acid alcohol 3 cc of conc HCl diluted to 100 cc. with 60% alcohol
3 Diluted methylene blue solution 10 cc of stock solution diluted to 100 cc. with distilled water (stock solution methylene blue 30 cc of saturated alcoholic methylene blue solution, 99 cc distilled water, 1 cc. of 1% potassium hydroxide).

Leprosy

Leprosy is an extremely chronic, infectious disease of a granulomatous nature which produces characteristic changes in the skin, nerves, bones, and internal organs and which is caused by Mycobacterium leprae.

The transmission is probably by direct contact with cases of leprosy

Incubation period three months to 20 years and more

The leprosy bacillus is closely related to the tubercle bacillus and corresponds to it in size, shape and staining qualities. The characteristic of Mycobacterium leprae is its intra and extracellular clumping in the form of globi or bundles. Causative organism (Plate XIII, upper half)

In tuberculoid leprosy, the demonstration of the bacteria is frequently difficult because the number of bacteria in this form is only small. In lepromatous leprosy, the bacteria are easily demonstrated in the nasal Diagnosis

smear. The nasal smear is generally made by scraping the membrane of the septum and transferring a little of this to a microscope slide for examination. Better results are obtained if the nostrils are dilated with a speculum, and a small curette specimen of the mucosa is obtained, (if necessary after local anaesthesia by the means of a Novocain-adrenaline swab). If the mucous membrane of the nose bleeds easily it should cause one to be suspicious. The material obtained is smeared on a slide,

made into the skin to the depth of the corium with a needle, the first few drops of blood wiped away, and the serum then issuing from the tissue should be transferred to an object slide. More precise is the excision of a small piece of tissue from the edge of a macule (if necessary, after local anaesthesia) which is then pressed out on a slide. It is also possible to examine blister fluid from blisters produced on suspicious sites by means of carbon dioxide snow or cantharides. The blister fluid is centrifuged and the deposit smeared onto a slide and stained in the usual manner

In lepromatous leprosy it is also possible to examine the layer of leucocytes in a tube of sedimented citrated or oxalated blood. A positive result is sometimes obtained here.

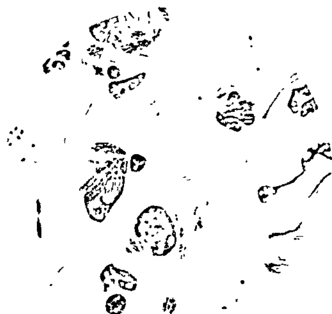
Staining — Leprosy bacilli are best stained with Ziehl-Neelsen method (see page 37). Leprosy bacilli, however, are less alcohol-fast than tubercle bacilli. It is advisable, therefore, in decolourising, to replace acid alcohol by 10—20% sulphuric acid.

Treatment — Contebén (see page 61) Neoteben (see page 66), diaminodiphenylsulphone (see page 61)

Streptococcal and Staphylococcal Infections

Causative organism (Plate XIV) — Streptococci are small round cocci which develop in the form of chains. In pus they are generally seen in very short chains in the form of diplococci. Staphylococci are somewhat smaller than streptococci and are found in the typical clusters of grapes

Diagnosis — Streptococci and staphylococci are found in pus of carbuncles, in the





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also be smeared. The skin should be made hyperaemic, a small puncture made into the skin to the depth of the corium with a needle, the first few drops of blood wiped away, and the serum then issuing from the tissue should be transferred to an object slide. More precise is the excision of a small piece of tissue from the edge of a macule (if necessary, after local anaesthesia) which is then pressed out on a slide. It is also possible to examine blister fluid from blisters produced on suspicious sites by means of carbon dioxide snow or cantharides. The blister fluid is centrifuged and the deposit smeared onto a slide and stained in the usual manner

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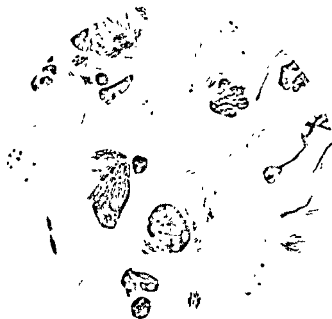
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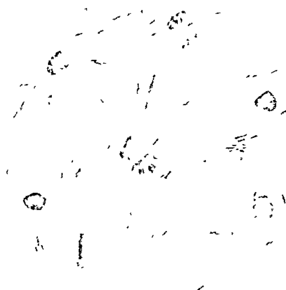
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Diagnosis Streptococci and staphylococci are found in pus of carbuncles, in the





blood in pyaemia and sepsis, and in the tissues in cellulitis, etc. These organisms are stained easily with all the basic aniline stains and are gram-positive. For differentiation of streptococci and staphylococci and numerous other similar small organisms, Gram's method is of great importance. This method depends upon the fact that by means of iodine all bacteria may be classified into two groups Those bacteria in which iodine deposits, and which do not give up this iodine when subsequently treated with alcohol are gram-positive. The gram-negative bacilli, however, lose this iodine deposit and thus are decolourised and take the subsequent contrast stain Apart from the staphylococci and streptococci the following organisms are gram-positive· M tuberculosis, M. leprae, B anthracis, C tetanus, diphtheria bacillus, gas gangrene bacilli, and pneumococci The following organisms are gram-negative· B. coli, V. cholerae, B dysenteriae, B. typhosus, H. influenzae, N. gonorrhoea, and N. meningococcus.

The dried and heat-fixed slide is stained for half-a-minute with carbol-gentian violet (10 cc. of a saturated solution of gentian violet in 96% alcohol plus 90 cc. of a 2 5—5% solution of phenol in distilled water) The stain should be filtered before use The stain is poured off the slide which is then covered for half-a-minute with dilute Lugol's solution (iodine 1, potassium iodide 2, distilled water to 300) The iodine solution is poured off and the slide is then decolourised with absolute or 96% alcohol, or with a mixture of alcohol and acetone in equal parts. This decolourisation is carried out until no further colour streams from the smear. Generally, this takes about one minute The slide is then thoroughly washed in water and counter-stained with 1—10 carbol fuchsin solution (see page 37) for 10—15 seconds It is then rewashed and allowed to dry Gram staining

Gonorrhœa

Gonorrhoea is an infectious venereal disease caused by gram-negative gonococci. The disease manifests itself by inflammatory changes of the mucosa, *especially of the genital mucosa, along which it spreads.*

Transmission is through sexual intercourse or by contact.

Incubation period 2—8 days

Causative organism (Plate XV, top half) The causative organism, Neisseria gonorrhoea is a diplococcus of coffee bean shape which is characteristically found intracellularly in polynuclear leucocytes.

Diagnosis In an acute urethritis a little of the pus is removed from the urine with a platinum loop and spread thinly on a microscope slide.

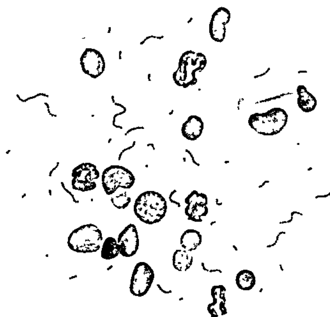
In chronic gonorrhoea the smear is made either of the pus which has collected in the course of the night or of the urethral threads which are found in the first morning sample of urine. These threads are generally found in the first portion of the morning urine and it is expedient to examine a small volume of urine of about 20—30 c.c. The urethral threads are removed from this with a pipette, and spread similarly to sputum preparations (between two slides). The threads contain, besides the leukocytes, masses of gonoccoci In chronic gonorrhoea it may be necessary to repeat the examination, if necessary following the repeated administration of provocative irritants.

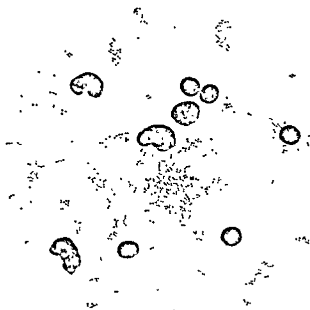
In women the urethral and cervical secretions should be used for examination The vaginal secretion is unsuitable because of the presence of numerous mixed organisms. In females, gonorrhoea generally spreads from the vagina to the anal mucosa. Material for examination may be taken here directly with a platinum loop. In vulvo-vaginitis of young girls, the diagnosis of gonorrhoea is easily made in the vulva secretion, because in this disease in young girls the vaginal mucosa is also affected

In blenorrhoeal conjunctivitis of infants and adults the demonstration of the causative organisms in the conjunctival secretion is often difficult because here gram-negative diplococci may be found which are similar to gonococci. In order to confirm the diagnosis in this disease, apart from the microscopical diagnosis, the case history and the clinical picture should be studied, and, when possible, a culture should be made.

Staining The smear is dried in the air or over a flame and stained without heating for a few seconds with dilute methylene blue solution (see page 37). After rinsing the slide with water, it is pressed dry between filter papers and finally dried high over a flame. In view of the heavy mixed flora of the female genitalia, Gram's stain should always be used for differential diagnosis.

Treatment Penicillin-depôt preparations (see page 66—67).





Chancre (Ulcus molle)

Chancre is an infectious venereal disease caused by specific organisms and clinically apparent by multiple ulceration of the skin and mucous membrane, usually the genital mucous membrane

Transmission is mainly by sexual intercourse

Incubation period 1—3 days.

The causative organism, Haemophilus ducreyi, is a non-motile, delicate, small, non-capsulating, non-sporing, gram-negative rod Because these organisms are frequently found in chains they have also been called strepto-bacilli. In order not to disturb this characteristic chain formation it is necessary to prepare the slides very carefully. The ulcer should be cleaned with physiological saline in order to remove the surface bacteria before a sample is taken for diagnosis After cleaning, a few tissue particles are scraped from the edge of the ulcer and smeared onto the microscope slide in a similar manner to a blood smear. Causative organism (Plate XV lower half)

All bacteria stains are suitable The simplest is methylene blue Staining

The typhus fevers (rickettsiasis)

The typhus fevers are virus diseases transmitted by lice or ticks The symptoms are continuous fever, encephalitis, exanthemata and other severe general manifestations Related to classical (or epidemic) typhus fever are also a number of other rickettsial diseases such as murine typhus, Rocky Mountain spotted fever, African tick typhus, tsutsugamushi, and others. The insects which transmit the disease are infected by taking up blood containing rickettsia from a sick patient within the first few days of the disease. The rickettsia, after multiplying for several days in the epithelial cells of the insect's stomach, are excreted with the faeces of the insect. Man is infected only by the rubbing of the insect's faeces into small scratches or by the direct inhalation of the dry faeces. The actual inoculation of the parasites by the insects through the intact skin is not possible.

Incubation period is on an average 12 days, with a minimum of 5 and a maximum of 23 days.

Rickettsia prowazeki or other rickettsiae Causative organism (Plate XVI upper half)

Chancre (Ulcus molle)

Chancre is an infectious venereal disease caused by specific organisms and clinically apparent by multiple ulceration of the skin and mucous membrane, usually the genital mucous membrane.
Transmission is mainly by sexual intercourse
Incubation period 1—3 days.
The causative organism, Haemophilus ducreyi, is a non-motile, delicate, small, non-capsulating, non-sporing, gram-negative rod. Because these organisms are frequently found in chains they have also been called strepto-bacilli In order not to disturb this characteristic chain formation it is necessary to prepare the slides very carefully. The ulcer should be cleaned with physiological saline in order to remove the surface bacteria before a sample is taken for diagnosis After cleaning, a few tissue particles are scraped from the edge of the ulcer and smeared onto the microscope slide in a similar manner to a blood smear. Causative organism (Plate XV, lower half)
All bacteria stains are suitable The simplest is methylene blue Staining

The typhus fevers (rickettsiasis)

The typhus fevers are virus diseases transmitted by lice or ticks. The symptoms are continuous fever, encephalitis, exanthemata and other severe general manifestations Related to classical (or epidemic) typhus fever are also a number of other rickettsial diseases such as murine typhus, Rocky Mountain spotted fever, African tick typhus, tsutsugamushi, and others. The insects which transmit the disease are infected by taking up blood containing rickettsia from a sick patient within the first few days of the disease The rickettsia, after multiplying for several days in the epithelial cells of the insect's stomach, are excreted with the faeces of the insect. Man is infected only by the rubbing of the insect's faeces into small scratches or by the direct inhalation of the dry faeces. The actual inoculation of the parasites by the insects through the intact skin is not possible.
Incubation period is on an average 12 days, with a minimum of 5 and a maximum of 23 days
Rickettsia prowazeki or other rickettsiae. Causative organism (Plate XVI, upper half)

Diagnosis The rickettsia cannot be demonstrated microscopically in the blood The picture in Plate XVI shows Rickettsia prowazeki and Rickettsia pediculi, the causative organism of Trench fever, in or on intestinal epithelium of an infected louse.

Treatment Chloramphenicol (see page 61) Light diet, milk, plenty of fruit juice, tea, etc.

Q-Fever

Q-fever is predominantly a pneumotropic systemic infection. Symptoms of influenza or typhoid generally appear together with a continuous or remittent fever generally of 4—12 days' duration

Causative organism The causative organism of Q-fever, Coxiella burneti, belongs to the rickettsial group of organisms.

Diagnosis The direct microscopical diagnosis in human blood has not, up to the present, been possible. By inoculation of human blood taken in the first few days of the disease into animals, or onto the membrane of hatching eggs, it is possible to culture the disease organisms Plate XVI, lower half, shows the typical form and arrangement of Coxiella in smears stained with Giemsa. The preparation is from the spleen of an intraperitoneally infected mouse

Treatment Chloramphenicol appears to be advisable here (see page 61)

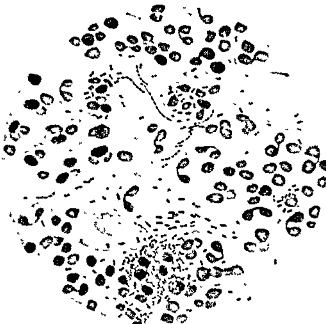
Trachoma

Trachoma is a chronic infectious disease of the conjunctiva and, occasionally, of the sclera and cornea caused by a virus. Both eyes are almost always simultaneously attacked. The disease commences with a diffuse inflammation of the conjunctiva and leads to the subepithelial follicular proliferation (trachoma follicles) The disease may progress to a necrotic breakdown of the trachoma follicles, the development of pannus and anomalies of the lid, resulting from scarring. Transmission occurs directly or indirectly through towels, linen, etc., from the infected to the healthy conjunctiva.

Causative organism (Plate XVII upper half) Trachoma is caused by a large microscopically visible virus

Diagnosis For the demonstration of the causative organism of trachoma, the inflamed conjunctiva should be carefully and lightly scraped with a spa-





tula The epithelial cells so obtained should be thinly spread on a fat-free microscope slide. Before scraping the conjunctiva, it is advisable to wipe away any secretion. After fixing the air-dried slide with methyl or ethyl alcohol, the smear is stained with Giemsa, preferably by the long process and with final differentiation (see page 8)

In conjunctival smears the characteristic intraplasma inclusion bodies are found (only sparsely) in the epithelial cells in various stages of development. The large inclusion bodies, lying alongside or surrounding the nucleus, contain numerous elementary bodies which stain red-violet with Giemsa Also present is a blue component in various shapes and varying amount. This is known as the basic substance, initial body, "plaques", etc. The elementary bodies which are about 400 mμ large lie singly, in twos or clumped in masses, and are also seen extracellularly They come from inclusion bodies either during the preparation of the slides (through destruction) or from the cells which have been killed as the result of the infection. Infrequently the elementary bodies, and even less frequently, the basic substance fragments may be seen in neutrophils, presumably being there through phagocytosis Because of the relative smallness of the elementary bodies, the use of an oil immersion lens is necessary for their demonstration The magnification of the microscope should not be less than 600—700 and it is important also to have a good source of light

Supronal Eye ointment (see page 72), chloramphenicol eye ointment (see page 61). In simple conjunctivitis Protargol Eye-Drops (see page 68) Treatment

The Pock Diseases

This group of virus infections commences with a prodromal stage of catarrhal changes in the mucous membranes of the nose and throat and with high fever. After generalisation, an erythematous or petechial rash on the skin and mucous membrane occurs This rash goes through a papular and finally a bullous stage The vesicles which become secondarily infected with bacteria, become full of pus, break down and lead to the characteristic widespread pustules

Transmission occurs very easily by contact or droplet infection

Incubation period 12—15 days

Variola major virus. Causative organism (Plate XVII lower half)

Diagnosis The [illegible]

[illegible]

occur which are known as Guarnieri's bodies

After experimental transmission of the human virus to the scarified rabbit cornea, intraplasmatic inclusion bodies develop in the corneal epithelium. The size of these inclusion bodies varies greatly. The young inclusion bodies are generally close to the nucleus of the epithelial cell. In later developmental stages the nucleus is deformed and finally surrounds the [illegible] inclusion body like a cap. In Guarnieri's bodies, reproduction of the virus probably occurs. Plate XVII, lower [illegible] cornea of a rabbit infected with [illegible]

[illegible]

Treatment bodies. For the suppression of the suppurative stage caused by the secondary infecting bacteria, [illegible]ronal (see page 71) or Badional (see page 57) [illegible]

Lymphogranuloma venereum

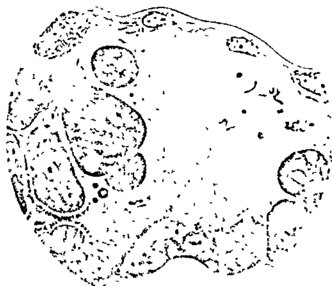
(Lymphopathia venereum)

(Climatic bubo)

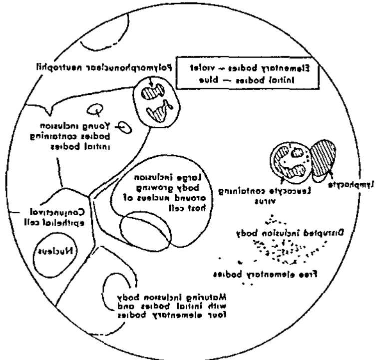
This is the so-called fourth venereal disease. Following a primary lesion on the genitals, an enlargement of the inguinal lymph glands occurs. Secondary infections produce cellulitis and necrosis. Rectal strictures as a result of perversion have been seen.

Incubation period 1—3 weeks

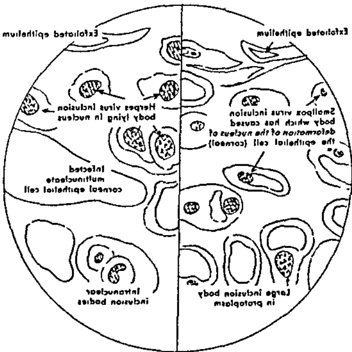
Causative organism (Plate XVII, upper half) The causative organism is related to the [illegible] of psittacosis and trachoma. It has the same development through intraplasmatic inclusion bodies as have these other organisms.







Trachoma virus, Conjunctiva smear
Giemsa staining, × 1100



Smallpox virus
Guarnieri's bodies
Rabbit cornea

Herpes virus
Lipschütz bodies
Rabbit cornea

Stain: Methylene blue – Phloxin
Magn. × 1100

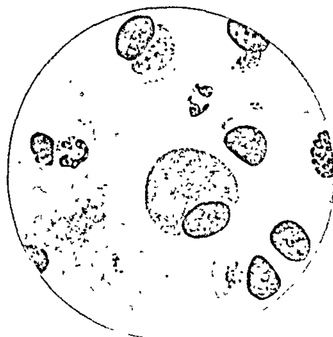




Plate XVIII, upper half, shows the typical picture of a Giemsa stained brain smear from a mouse which has been intracellularly inoculated with this virus. Free elementary bodies and inclusion bodies that have been mechanically liberated from the cell by the smear technique, as well as a cell filled with the virus, are to be seen *Diagnosis*

Fouadin (see page 62), Supronal and Solu-Supronal (see page 71) *Treatment*

Psittacosis

Psittacosis is a disease which, untreated, has a high mortality rate (20 to 40 %). This disease, with its severe systemic symptoms, presents the picture of an atypical pneumonia. Psittacosis is transmitted by parrots, parrakeets, and other birds such as sparrows, doves, chickens, etc. The disease is generally transmitted by inhalation of dust containing the virus

Incubation period 7—14 days.

The causative organism of psittacosis belongs to the group of large viruses stainable by Giemsa, to which lymphogranuloma and trachoma belong. These viruses are known as the psittacosis lymphogranuloma group The elementary bodies of these viruses are 250—400 mμ large, and, after staining, they may be seen with an ordinary microscope The demonstration of elementary bodies and inclusion bodies in material obtained directly from man is very difficult However, a large number of suitable experimental animals, especially mice, are available, and the virus may be grown on the chorioalantoic membrane of hens eggs *Causative organism (Plate XVIII, lower half)*

For the diagnosis of psittacosis virus, sputum is inoculated intraperitoneally into mice After about one week, the peritoneal epithelium contains the intraplasmatic inclusion bodies in all developmental stages, and, as well, a great number of free elementary bodies

Chloramphenicol (see page 61). *Treatment*

Helminth infections caused by nematodes

Filaria

The mature helminths live in lymphatic or in connective tissue According to their type, their site and the severity of the infection (together with the associated secondary infection) there develops a syndrome which

both in its general picture and in its details may differ markedly. Filaria, which are pathogenic to man, are viviparous. The embryos (microfilaria)

[illegible]

sexual maturity is achieved and the first microfilariae appear in the

[illegible]

who present no obvious clinical symptoms of the infection.

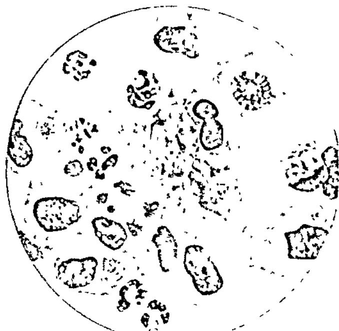
Plate XIX Diagnosis

For microscopical diagnosis only the microfilaria need be considered. These are small, highly motile, eel-shaped roundworms of about 0.25 mm in length. They are approximately as thick as the diameter of an erythrocyte. [illegible] appears in numbers in the blood at night. It is, therefore, essential that a blood sample should be taken at night for the diagnosis of this form of filaria. Other types have no definite periodicity and appear in large numbers throughout the day in the blood stream. Microfilaria of Onchocerca volvulus are demonstrated by puncture of any suspicious cutaneous node, or of the inguinal lymph glands. Another method is to remove a small piece of skin and allow it to remain for 2—3 hours in physiological saline. The microfilaria leave the skin and migrate in hundreds into the saline. Larvae of Onchocerca caecutiens can be demonstrated by a similar method. A staining method for microfilaria is given on pages 8 and 13.

Ancylostoma duodenale: Necator americanus

[illegible]

The adult worms produce ova which are excreted with the stool. At favourable temperatures and conditions of humidity, the ova develop outside the body to filariform larvae within five days. These larvae penetrate the skin, especially of barefooted persons. The migration of

the ancylostoma larvae is diagrammatically portrayed in Plate XX. T filariform larvae reach a blood vessel within 24 hours and finally arr in the lung after travelling through the systemic and pulmonary blo circulation. After a short period in the pulmonary capillaries, they th penetrate to the alveoli, then to the bronchi and trachea, and finally the oesophagus where they are then swallowed and reach the intestir The worm, which has developed during this migratory period, th attaches itself to the wall of the small intestine where it matures. Hoo worms may reach the age of seven years and more.

The microscopical diagnosis of hookworm infection depends upon t demonstration of ova in the stool. Ancylostoma ova are light grey, ov in shape, and are enclosed in a thin, smooth shell. The ova of Necat americanus are somewhat longer than those of Ancylostoma duodena and they are somewhat more tapered Generally, hookworm ova fresh stools are unfurrowed, they may, however, also contain 2– nuclei. The terminal stages of development are probably seen in stoo which have been standing for some time For the preparation of micr scopical specimens see pages 14—17.

Ascaridole-Bayer (see page 55) Bedermin (see page 59) Neobederm (see page 65).

Ascaris lumbricoides

Roundworms affect the general health by absorbing nutritive elemen present in the diet, and sometimes mechanically by causing an ileu The production of toxic, allergen-like substances by these worms indicated by the eosinophilia Adult roundworms can penetrate t gall-bladder and bile ducts. The larvae in the lung produce infiltratio which can be seen by X-ray. The ubiquitous roundworm is the most fr quent intestinal nematode parasitising man Children are especial affected.

The ova produced by the adult worm are excreted with the stool Th larvae are protected by a tough outer shell and thus remain infectio for many years in the ground When the ova are swallowed, the larva escape from the shell into the small intestine, they then penetrate th intestinal wall and gain entry into the blood circulation From here or

the ancylostoma larvae is diagrammatically portrayed in Plate XX. The filariform larvae reach a blood vessel within 24 hours and finally arrive in the lung after travelling through the systemic and pulmonary blood circulation. After a short period in the pulmonary capillaries, they then penetrate to the alveoli, then to the bronchi and trachea, and finally to the oesophagus where they are then swallowed and reach the intestine. The worm, which has developed during this migratory period, then attaches itself to the wall of the small intestine where it matures. Hookworms may reach the age of seven years and more

The microscopical diagnosis of hookworm infection depends upon the demonstration of ova in the stool. Ancylostoma ova are light grey, oval in shape, and are enclosed in a thin, smooth shell. The ova of Necator americanus are somewhat longer than those of Ancylostoma duodenale and they are somewhat more tapered Generally, hookworm ova in fresh stools are unfurrowed, they may, however, also contain 2—8 nuclei. The terminal stages of development are probably seen in stools which have been standing for some time For the preparation of microscopical specimens see pages 14—17. Diagnosis

Ascaridole-Bayer (see page 55) Bedermin (see page 59) Neobedermin (see page 65). Treatment

Ascaris lumbricoides

Roundworms affect the general health by absorbing nutritive elements present in the diet, and sometimes mechanically by causing an ileus. The production of toxic, allergen-like substances by these worms is indicated by the eosinophilia Adult roundworms can penetrate the gall bladder and bile ducts The larvae in the lung produce infiltrations which can be seen by X-ray. The ubiquitous roundworm is the most frequent intestinal nematode parasitising man Children are especially affected

The ova produced by the adult worm are excreted with the stool. The larvae are protected by a tough outer shell and thus remain infectious for many years in the ground When the ova are swallowed, the larvae escape from the shell into the small intestine; they then penetrate the intestinal wall and gain entry into the blood circulation From here on,

the migration of the worm and its further development is similar to that of the hookworm.

Diagnosis (Plate XXII, upper half) The adult worm which is as thick as a pencil, is about 40 cm. long and resembles the ordinary earthworm. The adult worm is occasionally seen in the stool; it is also sometimes vomited up, and has been known to make its exit from the body through the nostrils. The diagnosis of ascaridiasis does not depend upon the adult worm but upon the demonstration of the ova in the stool. For preparation of microscopical specimens see page 14—17.

Treatment Ascaridole »Bayer« (see page 55) Bedermin (see page 59) Neobedermin (see page 65)

Trichuris trichiura

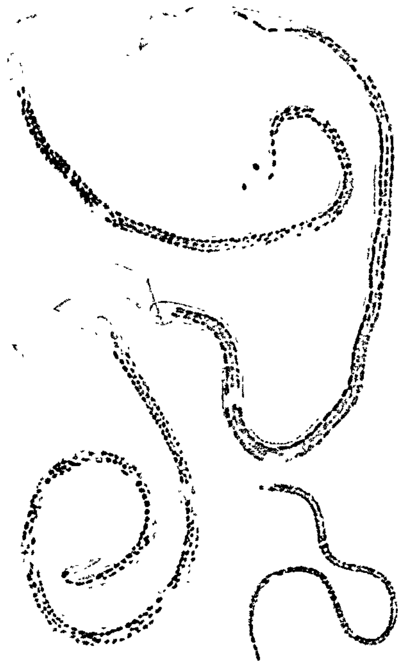
Clinical symptoms are not generally apparent. However, in severe infections abdominal pain, nausea, intestinal catarrh or constipation may occur. The transmission and migration in the body of this helminth is the same as with Ascaris lumbricoides.

Diagnosis (Plate XXI, upper half) The adult worm, which attaches itself to the mucous membrane of the large intestine, is practically never seen in the stool. Diagnosis depends *upon the demonstration of the ova. These are brown stained,* oval, lemon-shaped bodies. A characteristic of this parasite is the bulb-like or knob-like swelling at both ends of the ovum. For the preparation of microscopical specimens see page 14—17).

Enterobius (Oxyuris) vermicularis

The primary symptom of an infection with Enterobius is the anal irritation which itself can lead to ill-health. Infection and re-infection result from (1) scratching the irritated anal area with the fingers which then later transfer the ova to the mouth, (2) through swallowing of dust containing the ova — this especially applies to bedrooms, schoolrooms, etc., (3) by larvae which have hatched from the ova in the region of the anus, and which re-enter the anus and migrate to the intestine.

Diagnosis (Plate XXI, upper half) Ova are seldom seen in the stool. It is, therefore, not worthwhile to search for them. The female oxyuris creeps out from the anus, especially



at night, and lays its eggs in the external skin-folds surrounding the anus. The simplest method of diagnosis is by means of a cellophane strip A 7—8 cm. long cellophane strip, as can generally be purchased, is pressed onto the area of the anus, taken off immediately, and then laid upon a microscope slide. It is then examined under low-power in water or water plus toluene. The best time for detecting oxyuris ova is in the early morning before mechanical removal of the ova by dressing, defaecation, etc. If a cellophane strip is not available a thick test-tube, the round end of which has been roughened with sandpaper, is also suitable. The roughened end is moistened and this end is then rubbed for about ten seconds with a rotary movement in the region of the anus The debris at the end of the tube is then transferred to a microscope slide After this has been allowed to dry, it is examined under low power in cedar-wood oil without a cover glass The ova with their smooth surface, are oval and unsymmetrical (one long side is flat)

Atmonil (Badil) (see page 57), Butolan (see page 60)

Treatment

Helminth infections caused by Cestodes

Taenia solium, Taenia saginata, Diphyllobothrium latum, Hymenolepis nana

Persons infected with Taenia solium and Taenia saginata are often only aware of this when they first excrete segments of the worms Ravenous appetite alternating with anorexia, diarrhoea, abdominal pain, tiredness, and anaemia are sometimes seen as the result of an infection Diphyllobothrium infections may result in an anaemia very similar to pernicious anaemia. Hymenolepis infections when severe, especially in children, may produce psychic symptoms, apart from gastro-intestinal pain.

Intermediate hosts of Taenia saginata are beef-cattle, of Taenia solium, the pig, and for Diphyllobothrium, at first the water flea (Cyclops) and then fish. From the ova escape larvae which form cysticerci in the body musculature. Man is infected by eating beef, pork or fish in a raw or insufficiently cooked state. Hymenolepis nana has no intermediate host

Diagnosis (Plate XXI centre and lower half)

The segments of tapeworms (proglottides) which are excreted with the stool are the most obvious sign of an infection. With Taenia solium and Taenia saginata the female, pumpkin-seed-like, tapeworm segments which may be either in long or short chains, are always observed by the patient. In the mature proglottis the ova are present in large numbers. The proglottis also contains the fully developed embryo (onchosphere). The regular presence of ova in the stool is not to be expected with beef and pork tapeworm infections. The ova are round and enclosed by a thick shell possessing a radial striation. The ova of Taenia saginata are somewhat larger than those of Taenia solium. The differentiation of these two ova is only possible by actual measurement. The tapeworm segments are, in contra-distinction, easily differentiated by the number, and characteristic branching of the uterus. In Diphyllobothrium infections, however, the ova are found regularly in the stool, whilst the segments are rarely found. These ova, possessing a characteristic lid, are generally easily seen in the stool in wet preparations, their size being conspicuous (see page 13 and 9). In Hymenolepis nana infections, diagnosis is also made by finding the characteristic ova in the stool. If necessary, the concentration methods described on pages 14—16 may be used.

Treatment

Acranil (see pages 57—58) and Atebrin (see page 59).

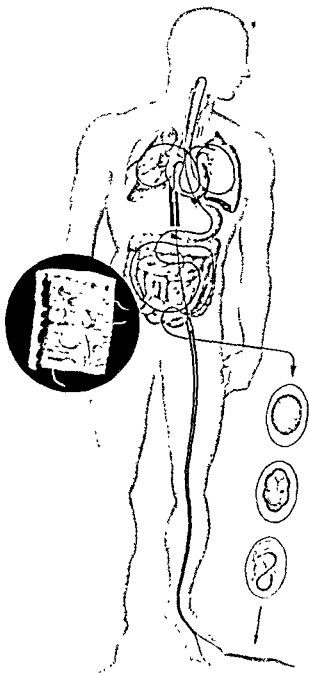
Helminth infections caused by Trematodes

Schistosomae (Bilharziae)

(Schistosoma mansoni, Schistosoma haematobium, Schistosoma japonicum)

Plates XXIII and XXIV

The infections with the above parasites are known as schistosomiasis. The mature helminths are found coupled in the veins of the abdominal organs and of the small pelvis. The ova produced by the female schistosome penetrate the walls of the blood vessels into the surrounding tissue and from there they enter the mucous membrane of the bladder and large intestine.



Trichuris trichiura

Ancylostoma duodenale

Enterobius vermicularis

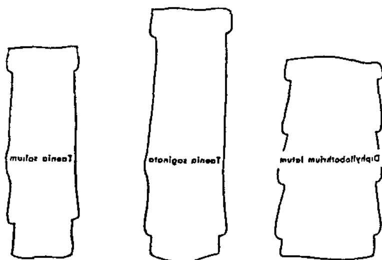
Diphyllobothrium latum

Taenia saginata and solium

Hymenolepis nana

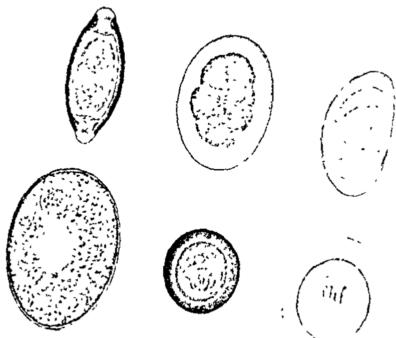
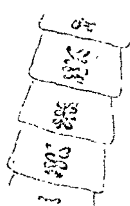
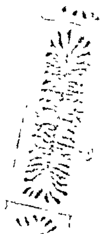
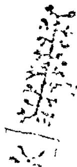
Helminth ova

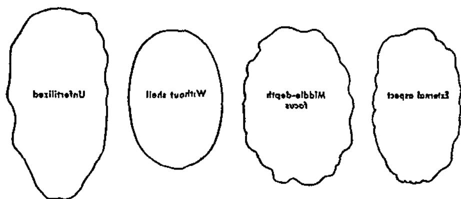
Magn. x 650



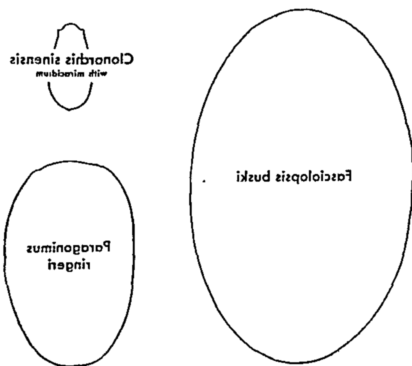
Tapeworm segments

Magn. x 3



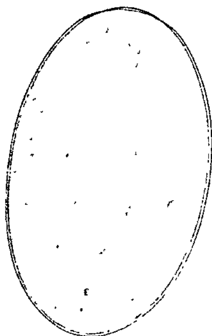
Ascaris lumbricoides ova
Magn. x 650



Helminth ova
Magn. x 650







This migration produces inflammatory and proliferative changes The ova are excreted with the stool or urine, and should they be passed into water the miracidia hatch out and parasitize various types of snail An enormous multiplication occurs in these intermediate hosts After reaching a certain stage of development, the parasites leave the snail as fork-tailed cercariae and exist in their watery environment The cercariae can penetrate the intact skin of man, and do so when man bathes or works in water containing them. The development cycle is shown in Plate XXIII. Plate XXIV shows male and female S. mansoni in the characteristic coupled state (the female in the gynaecophoric canal of the male).

Diagnosis (Plate XXV)

The microscopical diagnosis of schistosomiasis depends on finding the ova or the hatched larvae (miracidiae). This can sometimes be very difficult and may require repeated examinations The ova of Schistosoma haematobium are found in the urine sediment, those of Schistosoma mansoni and japonicum in the stool If bloody mucous flakes are present, the ova may frequently be found upon direct examination of these, otherwise the sedimentation or centrifugation techniques given on page 14, and the concentration method of Telemann given on page 16 should be used. An important diagnostic technique is the miracidia hatching test given on page 14. A further important method for the demonstration of ova, especially in infections with mansoni and japonicum but also in the presence of haematobium, is rectal biopsy By means of a proctoscope the mucous membrane is examined, and with a special forceps a small piece of the mucosa and submucosa (about the size of a rice grain) is taken from the right plica transversalis of the rectum. This mucosal particle is placed in water for about 5 minutes to free it from blood pigments and is then pressed between glass until one is able to see through it. Schistosoma ova may then be clearly seen In Schistosoma haematobium infections, the mucous membrane of the bladder may be examined by means of the cystoscope and, if necessary a biopsy may be taken In all examinatons, it is important to differentiate between viable and dead ova In doubtful cases the miracidia hatching test will give a definite answer Plate XXV shows the viable ova of the three types of schistosome.

Treatment

Fouadin (see page 62). Miracil D (see page 63)

Clonorchis sinensis

An infection with Clonorchis sinensis, and the related parasite Opisthorchis felineus, produces a chronic disease of the bile duct, enlargement of the liver, jaundice, and chronic diarrhoea.

The feeding of fish raised for human consumption on human excrement, or the fertilisation of water plants with this material leads to the infection of the snail which acts as the intermediate host. From these intermediate hosts, develop cercariae which bore into the skin of fresh water fish. Man is infected by eating these fish in a raw or insufficiently cooked state

Diagnosis (Plate XXII centre) The diagnosis of clonorchiasis depends upon the demonstration of the ova in the stool. The small eggs which are flask-like and tapered towards one end, possess a characteristic lid. In the duodenal fluid, these ova may often be found with greater certainty than in the stool.

Treatment Fouadin (see page 62).

Fasciolopsis buski

Fasciolopsis buski is, in East Asia, of definite pathogenic importance. The infection results from eating water plants on which the cercariae have encysted. The clinical picture is that of a more or less developed disturbance of the digestive system; secondary anaemia and pyschic disturbances have also been observed. Children suffer from a lack of growth and development, when parasitized by this fluke

Diagnosis (Plate XXII lower half) The large ova are easily demonstrated in the stool.

Paragonimus ringeri (westermanni)

Infections with Paragonimus ringeri, lung fluke, have been reported from East Asia and West Africa. The infection results from the consumption of fresh water crabs in which the cercariae have encysted. The larvae are released (in the intestinal tract), and migrate through the intestinal wall into the abdominal cavity. The majority then penetrate the diaphragm and enter the lung where, in the neighbourhood of the bronchi, they form colonies of cysts.

Diagnosis (Plate XXII ...lf) Paragonimus sputum is red-brown, sometimes bloody and contains the ova of the parasite. As well as in sputum, the ova are frequently found in the stool, appearing there from the swallowed sputum.

List of stains and reagents used

A. Standard stain solutions
- Giemsa
- Haematoxylin
  - a) Delafield
  - b) Heidenhain
- Carbol fuchsin (Ziehl-Neelsen)
- Carbol gentian violet for Gram stain
- Methylene blue

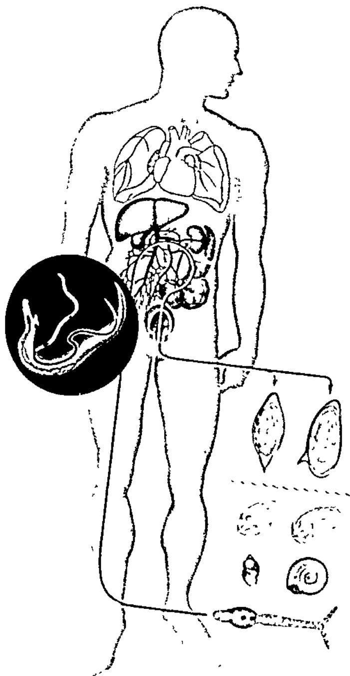
B. Standardised stains for preparing solutions
- Eosin
- Fuchsin
- Gentian violet
- Giemsa powder
- Haematoxylin
- Light green
- Methylene blue
- Methyl blue
- Orange G
- Acid fuchsin

C. Auxiliaries
- Caedax, neutral mounting medium to replace Canada balsam
- Canada balsam for mounting histological specimens
- Immersion Oil, synthetic
- Liquefac, for liquefying sputum for tubercle bacilli examination
- Phenol, cryst.
- HCl alcohol 3% — for decolourisation of non-acid fast bacteria
- Weise's Buffer, for preparation of buffered water for various staining methods

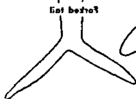
The above »Bayer« stains, stain solutions and auxiliaries as well as many others, can be obtained through the firm
SERUM-VERTRIEB MARBURG G m b H, Marburg/Lahn, Germany

List of Recommended Products

Name and Composition	Administration and Dosage
Acranil	**Giardiasis (lambliasis)**
Chloromethoxy-acridylamino-diethylaminopropanol-dihydrochloride	Adults and children over 8 years: 1 tablet three times daily; children 4—8, 1 tablet twice daily, children under 4, one tablet daily. The tablets should be swallowed whole, and taken after meals Duration of treatment 5 days.
	Tapeworm
	On the day before the treatment the midday meal should be rich in protein and poor in carbohydrate No evening meal; a purgative (castor oil or saline) should be taken instead. The following morning, fasting, Luminal (adults 0 1 g., children 8—12, 0 05 g., children 4—7, 0 025 g.). Then after half-an-hour, — Adults, 3 Acranil tablets, then 15 minutes later another 3 tablets, then 15 minutes later 2 tablets Total 8 tablets of 0 1 g Children 8—12 years, 2, then 2, then 2, at the same 15 minute intervals Total 6 tablets. Children 4—7 years, 2, then 2, as above Total 4 tablets. Tablets are swallowed whole with half a glass of water to which a knife-point of sodium bicarbonate is added to improve gastrointestinal tolerance to medicament. Two hours after last Acranil dose, a strong purgative (castor oil or magnesium sulphate) should be taken. As soon as the purgative has acted (the dose should be repeated if no bowel action occurs) food may be taken If revulsion is experienced at the prospect of taking the purgative, nausea and vomiting can be avoided by giving another dose of Luminal (dosage as above) beforehand.

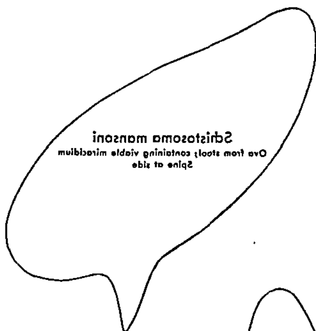


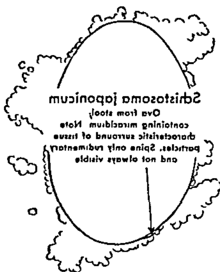
Schistosoma mansoni
Coupled worms
Giemsa staining, magn. x 25

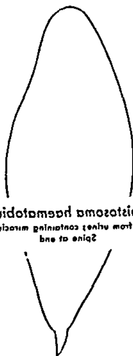


Schistosoma mansoni
Cercaria
Cresyl violet staining, magn. x 200









Schistosoma ova
Wet preparation
Magn. X 650



Name and Composition	Administration and Dosage
Acranil (continuation)	**Tapeworm** (continuation)
	If it is possible to administer Acranil by means of a duodenal tube the following doses should be given Men 8, women 6, children 4—5 tablets, dissolved in 100, 80 and 60 c.c. respectively of hot water (the sugar-coated tablets should be cut into several parts previously) The solution is cooled and filtered and given through the tube the tip of which should be about 70 cm past the pylorus The tube may be left in position and the purgative administered through this later. Premedication and other pre and post treatment conditions are identical with those described for non-intubation
	Packings 15 and 250 sugar-coated tablets of 0.1 g
Ascaridole capsules Pure ascaridole 0.3 g (effective component of chenopodium oil) in gelatine capsules.	**Ascaridiasis and Ancylostomiasis** After fasting from the previous evening, adults take, on an empty stomach the following morning 2 capsules, then a purgative Food may be taken only after the purgative has acted Treatment may be repeated only after 14 days have elapsed Children under 18 receive only diluted ascaridole as the 2.5% solution in castor oil (see below).
	Packings Various sizes for capsules of 0.3 g
Ascaridole solution «Bayer» 2.5% solution of pure ascaridole in castor oil	**Ascaridiasis and Ancylostomiasis** After fasting from the previous evening, adults take on an empty stomach the following morning 2.5 c.c., children up to 12 years 1 c.c./year of age, adolescents from 13—18 years, 1¼ c.c. year of age These quantities are taken in one dose If purgation has not occurred within 4 hours another dose of purgative only, must be taken (castor oil or saline) Food may be taken only after the purgative has acted Treatment may be repeated only after 14 days have elapsed
	Packings: 15 c.c. and 25 c.c.

Name and Composition	Administration and Dosage
Atebrin 2-methoxy-6-chloro-9-α-diethylamino-δ-pentylamino acridine dihydrochloride	**Malaria** Therapy: Adults and well-developed children over 8 years, 3 x 1 tablet daily; children 4—8 years, 2 x 1 tablet daily; children under 4 years, half a tablet twice daily. In order to achieve an effective blood-level rapidly this dose may be doubled on the first day. Duration 4—7 days. Tablets should be taken with meals and swallowed with plenty of water. Prophylaxis: Adults and children over 4 years, 4 tablets weekly; children 2—4 years, 2 tablets; children under 2 years, 1 tablet. The weekly dose should be divided and given on two successive days
	Giardiasis (lambliasis) Dosage and administration as for Acranil. One tablet of Atebrin is equivalent to one tablet of Acranil
	Tapeworm Either as tablets or through the duodenal tube, dosage as for Acranil One tablet of Atebrin is equivalent to one tablet of Acranil
	Lupus erythematosus Commence as for malaria (above), and continue for at least 7 days. Continue with 1 tablet daily (adults), for several weeks until inflammatory processes have disappeared It is necessary for the success of the treatment that the skin should take a definite yellow tint.
Packings	15 and 300 tablets of 0.1 g.
Atebrin for injection Atebrin-dimethanesulphonate. The dose declared on the label of the ampoule is calculated as that of Atebrin dihydrochloride	**Malaria** For intramuscular administration 0.1 g is dissolved in 2 c.c. of water (or 0.3 g in 5 c.c.) Maximum single dose for adults and well-developed children over 8 years, 0.3 g, for children from 5—8 years, 0.2 g; children from 1—4 years, 0.1 g; children under 1 year 0.05 g In debilitated patients, especially children, give smaller doses

Name and Composition	Administration and Dosage
Atebrin (continuation)	**Malaria** (continuation) For intravenous administration Adults 0.1 g dissolved in at least 3 c.c. of water. This dose can be repeated several times during the day. Children correspondingly less. Oral treatment should be instituted as soon as possible. In calculating the total dose necessary for a course of treatment, the doses given for tablets are applicable
	Oriental Boil (Cutaneous leishmaniasis) Injections of 1—2 c.c. of a 10—20 % solution under or around the single boils.
	Packings. 6 ampoules of 0.1 g 2 ampoules of 0.3 g
Atmonil Pure crystal violet (hexamethyl p-rosaniline chloride) in an enteric-coated tablet	**Oxyuriasis** Adults. 3 x 3 tablets daily, children, for every 2 years 1 tablet daily. Duration 7 days. The dose for children should be divided and given with the meals. The tablets are swallowed whole with a little water at the commencement of meals. During the course of treatment only food which is easy to digest should be taken. Enemas are unnecessary. Daily bowel movements should be assisted by a gentle laxative. Personal cleanliness (of hands, etc.) should be scrupulous to avoid reinfection. Should reinfection occur repeat course of treatment
	Packings. of 35 and 63 enteric coated tablets of 0.02 g
Badional p aminobenzene-sulphothiocarbamide	**Plague, Smallpox, Tropical Ulcer** For the first two to three days of treatment the following average daily doses should be given Infants, 0.15—0.2 g./kg Children 1—3 years, 0.15—0.2 g./kg Children 4—6 years, 0.1—0.15 g./kg older children, 0.075—0.1 g./kg Adults 0.075 g./kg Whether given orally or intravenously these doses should be given as 3—5 single divided doses.

Name and Composition	Administration and Dosage
Bayer 205 (Germanin) (continuation)	**Trypanosomiasis** (continuation) Therapy. Single doses of 1 g. In robust individuals this may be increased up to 1.5 g. to 2 g. For children 0.2 g—0.75 g., according to age. The total dose for a curative course is at least 5 g. for adults. This may be raised to 10 g. if the clinical picture requires it. The injections should be given at 5-day-intervals. If the C.S.F. is positive for trypanosomes, Bayer 205 should be combined with tryparsamide. For example, 3—5 injections of Bayer 205 followed by 5 injections of tryparsamide at 5-day-intervals. **Pemphigus and Dermatitis herpetiformis** Adults: First day 0.25 g. then slowly increasing (under careful clinical observation) up to 0.5 g. (maximum of 1.0 g.). Total dose 5 g., given at 2-day intervals (1 g. doses at greater intervals).

All injections are given as a 10% solution, intravenously.

Packings: 10 ampoules of 0.5 g.
5 ampoules of 1.0 g.

Bedermin Capsules A stabilised mixture of ascaridole (1 part) and carbon tetrachloride (6 parts) in gelatine capsules containing 0.6 g.	**Ancylostomiasis and Ascaridiasis** After fasting from the previous evening, adults take, on an empty stomach the following morning 6 capsules; children over 16 years, 5 capsules, children over 14, 4 capsules. After this, a purgative is taken and when this has acted food may be eaten. The treatment may not be repeated before 14 days have elapsed. Children under 14 years should be given Bedermin only as the 14% solution in castor oil (see under).

Packings: 6 and 50 capsules of 0.6 g.

Bedermin Solution A 14% solution of Bedermin in castor-oil	**Ancylostomiasis and Ascaridiasis** After fasting from the previous evening, children take, on an empty stomach, the next morning the following doses: up to 12 years of age, 1 c.c. per year of age; older children 1¼ c.c. per year of age, adults over 18 years, 25 c.c.

Name and Composition	Administration and Dosage
Bedermin Solution (continuation)	Ancylostomiasis and Ascaridiasis (continuation)
	If the bowels have not acted within 4 hours as a result of the castor oil in Bedermin Solution, an additional purgative must be taken (castor oil or saline) Food should be eaten only after the purgative has acted. Treatment should not be repeated before 14 days have elapsed.
Packings:	Bottles of 25 c.c.
Butolan	Oxyuriasis
Carbaminic acid ester of p-oxydiphenylmethane	Adults and children over 10 years 3 x 1 tablet daily Children half a tablet thrice daily; infants one-quarter tablet thrice daily. Duration 7 days. Daily bowel movement should be promoted by means of a laxative. Special care should be paid to personal hygiene (cleanliness of hands, etc.) to avoid reinfection. If reinfection occurs, repeat course.
Packings:	20 tablets of 0.5 g
Campolon	Anaemias of all types
Injectable liver extract 1 c c contains 6 mcg. of vitamin B_{12} (determined microbiologically)	For blood regeneration, especially after malaria, hookworm diseases, and other infectious diseases Adults 2 c.c. or more, intramuscularly of either preparation. This dose is given daily or at longer intervals, according to the severity of the case
Campolon forte	
1 c.c. contains 10 mcg. of vitamin B_{12}	
Campoferron	
Contains the blood forming principles of the liver prepared by the Campolon process, with the addition of 0.25 % iron and 0 003 % copper	Adults, 5—10 c.c. (1—2 teaspoons), two or three times daily, orally. Children correspondingly less. Packings. Campolon, 5 ampoules of 2 c.c. 3 ampoules of 5 c.c. Campolon forte, 5 ampoules of 2 c.c. Campoferron, bottles of 100 c.c.

Name and Composition	Administration and Dosage
Chloramphenicol d(-)threo-1-p-nitrophenyl-2-dichloracetylamino-propane 1,3-diol	**Typhus, Psittacosis, Q-Fever** Orally, adults take an average daily dose o 15—30 mg./kg; children 25—50 mg./kg., divided int 3—4 single doses **Trachoma** Used locally as eye-ointment. Treat conjunctiva once or twice daily with 1 % chloramphenicol in water miscible ointment base
Conteben p-acetylaminobenzaldehyde thiosemicarbazone	**Leprosy** Commence with 25—50 mg daily. Increase dose according to type of leprosy, stage of disease, and tolerance of patient to drug to 100 mg daily with a maximum dose of 150 mg. daily. Children less. Duration of treatment depends upon clinical and bacteriological progress Packings: Tablets of 0.025 g and 0.05 g
Dapsone aminodiphenyl-ulphone (DDS)	**Leprosy** Daily oral dose of 25 mg for six days of week. Slowly increasing to 100 mg daily. Intramuscular depôt treatment by weekly injections of 150 mg to 600 mg in a watery or oily suspension. Children less, according to age. Duration of treatment, and treatment free intervals depend upon the toleration and clinical effect

Name and Composition	Administration and Dosage
Fouadin (Neo-Antimosan) Sodium salt of antimony^{III}-pyrocatechol sodiumdisulphonate Content of trivalent antimony 13.5 % 1 c c of the 6.3 % solution contains 8 5 mg of antimony^{III}	Schistosomiasis Adults: Commencing dose 3 5 c.c., further doses 5 c.c. Children: Commencing dose 0.5 c.c./10 kg bodyweight, further doses 1 c.c./10 kg. bodyweight First three injections daily, then every second day. Total 10—15 injections. Intensive course (after Halawani, Cairo); with strict control of renal function, hospitalized patients receive (twice daily) for 4 days, the above doses. Well-developed out-patients receive once daily for 10 days, the above doses Lymphogranuloma venereum – Granuloma inguinale. Oriental Boil (cutaneous leishmaniasis) Espundia (mucosal leishmaniasis) Infections with Clonorchis sinensis Single doses as for schistosomiasis First three injections daily, further injections also daily if well-tolerated, otherwise every other day or at longer intervals Total number of injections, 15—20 Trichinosis Single doses as for schistosomiasis Total 4—6 injections at intervals of 1—2 days Packings: Boxes of 1 x 0 5 c.c, 1 x 1.5 c.c and 8 x 3.5 c.c. Boxes of 1 x 0.5 c.c., 1 x 1.5 c c. and 13 x 3.5 c.c. Boxes of 1 x 3.5 c c. and 9 x 5 c.c. Boxes of 1 x 3.5 c.c. and 14 x 5 c c. Bottles of 25 c.c. and 50 c.c. Powder in bottles of 500 g and 1000 g
Marfanil-Prontalbin Powder Marfanil is 4-amino-methylbenzenesulphonamide hydrochloride. Prontalbin is 4-aminobenzene sulphonamide The mixture is in the proportion of Marfanil 1 part, Prontalbin 9 parts.	Tropical Ulcer and Infected Wounds In support of surgical measures, the wound is dusted with the powder and then bandaged. To prevent the bandage sticking to the wound the covering dressing should be thinly smeared with vaseline Packings· Sprinkler tins of 10 g and 50 g

Miracil D

1-diethylamino-ethylamino-4-methyl-thioxanthone hydrochloride

Miracil D comp.

1-diethylaminoethyl-amino-4 methyl-thioxanthone-hydrochloride plus 0.009 % belladonna alkaloids

Schistosomiasis (haematobium and mansoni)

For complete eradication of the parasites the following doses are considered necessary

S. mansoni and S. haematobium infections in the Middle East (Egypt, Arabia, etc.) 200 mg/kg bodyweight

S. mansoni infections in South America at least 100 mg./kg

Schistosome infections with Equatorial African and South African strains 60—100 mg./kg

Single dose [illegible]

[illegible] course

Adults and children over 12 1 tablet thrice daily

Children from 6—12 years old 1 tablet twice daily

Children from 1—6 years old 1 tablet daily

Duration of treatment Middle East 20 days [illegible]

Intensive course

Bodyweight kg	No. tablets daily
60 and over	6
50	5
40	4
30	3
20 and under	2

Duration of treatment 5—10 days. This intensive course is the most effective, but because of the higher daily dose the side-effects are more pronounced, though they are quite harmless.

Increasing and decreasing course

Bodyweight kg	Daily number of tablets Day 1 2 3 4 5 6 7 8 9 10	Total number of tablets
60 and over	1 2 3 4 5 5 4 3 2 1	30
50	1 2 3 4 5 4 3 2 1	25
40	1 2 3 4 4 3 2 1	20
30	1 2 3 3 3 2 1	15
20 and under	1 1 2 2 2 1 1	10

Name and Composition	Administration and Dosage
Miracil D comp (continuation)	Schistosomiasis (haematobium and mansoni) (continuation)

This course has shown itself to be particularly well tolerated in South America.

Miracil D and Miracil D comp. tablets are sugar coated to facilitate swallowing. They should be swallowed whole, directly after meals. The daily dose should be taken as three to four single doses

Side-effects When administered in high doses, a number of side-effects may occur, such as abdomi-*nal discomfort, anorexia, and possibly vomiting In* children these effects are seldom seen. With daily doses of 3 x 1 tablets daily (children correspondingly less) side-effects are only infrequent. The belladonna added to the Miracil D comp tablets also increases their tolerability considerably If abdominal discomfort does occur, it is usually related to an empty stomach, and some light, easily-digestible food should be eaten between the main meals to overcome the side-effects. If the side-effects cause a severer upset, the course should be discontinued for a *day or two until the symptoms subside, when it may* be commenced again

During the course of treatment a temporary yellow discolouration of the skin occurs This is due to the colour of Miracil itself, and it disappears rapidly after treatment. It is in no way connected with any organic damage.

Packings: Miracil D. 30 and 1000 sugar-coated, yellow tablets of 0 2 g.

Miracil D comp 30 and 1000 sugar-coated green tablets of 0.2 g

eo-Bedermin

Ancylostomiasis, Ascaridiasis, Oxyuriasis

rabilized combination
f ascaridole and
etrachlorethylene in the
roportion of
6 by weight
elatin capsules
f 0.35 g
4% solution
n castor oil.

In ancylostomiasis, ascaridiasis, oxyuriasis after fasting from the previous evening the following doses are taken, fasting, the next morning

Age in years	Number of capsules	Number of measures or graduations on the flask
1		ca ½
2 — 3	1	1
4 — 5	2	2
6 — 7	3	3
8 — 9	4	4
10—11	5	5
12—13	6	6
14	7	7
15	8	8
16	9	9
17	10	10
18	11	11
over 18	12	12

Children having a particularly healthy constitution may take a dose corresponding to that of the next year of age Undernourished and underdeveloped persons of every age, however, are given one capsule or measure less than indicated in the table

The capsules are swallowed whole, followed by a little water A strong aperient (castor oil is most suitable) is taken half an-hour later

When Neo-Bedermin solution has been administered, no aperient is necessary since the solution contains castor oil

After taking the capsules or solution no food should be eaten for the next four hours. If the bowels have not acted within this time, a purgative must be taken Solid food may only be taken after the termination of the course

An aperient should also be administered on the day following the course, to remove the destroyed or affected worms still remaining

The course must not be repeated until 14 days have elapsed

Packings: Capsules of 0.35 g Bottles of 12 and 100
14% solution Bottles of 30 c.c.

Name and Composition	Administration and Dosage

Neostibosan

Complex compound of p-aminophenylstibinic acid-p-acetylamino phenylstibinic acid, and antimony diethylamine. The complex contains 42—43 % of pentavalent antimony

Kala-azar

Intramuscularly as a 25 % solution; intravenously as a 5—10 % solution, in the following doses:

	Commencing dose	Later doses
Infants	0.05 g	0.1 g
Children 2— 4	0.05—0.1 g.	0.2 g
Children 5— 9	0.1 —0.2 g	0.25 g.
Children 10—15	0.2 g.	0.3 g.
Adults	0.2 g	0.3—0.45 g

Total dose 8—12 injections at intervals of 2—3 days
Short intensive course: 8 daily injections of the doses given above.

Packings: Ampoules of 0.05 g, 0.1 g, 0.2 g and 0.3 g For mass treatment, ampoules of 1 g, 2 g and 3 g.

Neoteben

Isonicotinic-acid-hydrazide

Leprosy

Dosage:

Bodyweight kg	Commencing dose 2.5 mg /kg/day	Average dose 5 mg /kg /day	Maximum dose 7.5 mg./kg./day
20	50 mg.	100 mg.	150 mg
30	75 mg	150 mg	225 mg.
40	100 mg.	200 mg	300 mg
50	125 mg.	250 mg.	375 mg
60	150 mg	300 mg	450 mg.

The daily dose should be divided into 2—3 single doses. Duration of treatment, and treatment-free intervals, depend upon the toleration and the clinical effect.

Packings: tablets of 0.05 g, 0.1 g. and 0.2 g.

Penicillin Depot-Preparations

Procaine penicillin in oil (PAM)

Yaws, Syphilis, Relapsing Fever, Gonorrhœa

Procaine-penicillin in oil (PAM) contains 300,000 I U/c.c. of crystalline procaine penicillin G plus 2% aluminium monostearate.

Packings: vials of 10 c.c. (300,000 I U/c.c.)

Name and Composition	Administration and Dosage
Rapidocillin	Rapidocillin contains 300,000 I U of crystalline procaine penicillin G in suspension, and 100,000 I. U of crystalline penicillin G-potassium in solution, per 1 c.c. ready-to-inject solution Packings: Vials of 400,000 I U. + 1 ampoule of 2 c.c. sterile redist water. Vials of 2,000,000 I. U + 1 ampoule of 5 c.c. sterile redist water
Periston N Periston N is a 6% solution of polyvinylpyrrolidone of an average molecular weight of 12,600, in physiological saline	Cholera To eliminate the bacterial toxins. Adults 100 c. c. and more; children up to 100 c.c., daily Periston N should be given slowly by intravenous infusion. Packings Infusion bottles of 100 c c.
Plasmoquine N-diethylamino-isopentyl-8-amino-6-methoxyquinoline as the methylene bis B-hydroxynaphthoic acid salt The dose declared on the label of the ampoule is calculated as that of plasmoquine hydrochloride	Malaria To conclude a Resochin or Atebrin course, adults and children over 8 years receive 2—3 tablets daily; children 4—8, 1—2 tablets daily; children under 4, ½—1 tablet daily Duration of treatment depends upon its purpose To destroy falciparum gametocytes, a one to two-day-course is sufficient To prevent relapses in vivax and quartan malaria, the curative effect increases in proportion with the length of treatment A course of 14—21 days gives the best results
Plasmoquine for injection A 1% solution of plasmoquine hydrochloride	1 c.c. of Plasmoquine for injection is equivalent to 1 tablet Thus in the above dosage scheme for Plasmoquine tablets, Plasmoquine for injection may be considered as either partly or wholly replacing the tablets Packings 15 and 500 tablets of 0.01 g 10 ampoules of 1 c.c. of a 1% solution 10 ampoules of 3 c.c. of a 1% solution

Name and Composition	Administration and Dosage
Primaquine Bayer 8-(4-amino-1-methylbutylamino)-6-methoxyquinoline diphosphate. The dose declared on the label is calculated as that of the base	*Malaria* 1. For relapse-free cure of vivax malaria and quartan malaria. Treat acute attack with Resochin (see below) Simultaneously, or directly after the Resochin course, give a 14—15-day-course of primaquine. Adults and children over 8 years, 1 tablet daily; children over 4 years, half-a-tablet daily; children under 4 years, one-third to one-quarter tablet daily Tablets should be taken with plenty of fluid after meals. 2 For the safe termination of clinical prophylaxis. After the malarial area has been left, the weekly Resochin dose (see below) should be continued for 14 days During this period primaquine should be taken as given under the first heading. 3. For the destruction of gametocytes especially in falciparum malaria A three-day-course of treatment with the above doses is sufficient. Packings. 15 tablets of 15 mg.
Protargol Eye-Drops 3% stable solution of protargol (silver proteinate)	*Conjuctivitis* One or more drops are instilled into the eye mornings and evenings. Packings. Bottles of 20 c.c.
Resochin 7-chloro-4-(4'-diethylamino-1-methyl-butylamino)-quinoline diphosphate	*Malaria* Therapy. A One-day-treatment of an attack of malaria occurring in the indigenous population of the malarial area (partially immune patients)

Adults and children over 12	4—6 tablets
Children 10—12	3—5 tablets
Children 6—9	3—4 tablets
Children 2—5	2—3 tablets
Children 1—2	1—2 tablets
Infants	1 tablet

Name and Composition — **Administration and Dosage**

Resochin (continuation)

Malaria
(continuation)

B. Two-day-treatment of a malaria attack in non-immunes (that is people who have not grown up in and are not permanently resident in the malarial area, and who thus have not developed a partial immunity to the locally prevalent parasite strains e.g., Europeans in the tropics, soldiers in foreign territories, workers on roads, railways, etc) For these patients a two-days treatment is advisable, as follows:—

Age	Tablets 1st day	Tablets 2nd day	Total
Adults and children over 12	6	4	10
10—12	5	3	8
6—9	4	2	6
2—5	3	1	4
1—2	2	1	3
Infants	1	1	2

The daily dose may be taken all at once or divided into 2—3 single doses. The tablets should always be taken after meals.

Prophylaxis. Suppressive treatment

The tablets are always taken on the same day of the week. Adults and children over 10 years take 2 tablets, children under 10 years, 1 tablet. Tablets are taken after meals. In the first week of treatment the dose should be doubled. After leaving the malarial area, the prophylaxis should be continued for a fortnight, and then terminated — with a single-dose treatment (see under B 1 day). For the indigenous inhabitants of a malarial area — the partially immunes, one tablet weekly is sufficient.

Packings 10, 100 and 1,000 tablets of 0.25 g

Name and Composition	Administration and Dosage
Resochin 5% solution	Resochin solution when given intramuscularly is well tolerated and causes no local reaction. Intravenously, Resochin must be given slowly (15—20 seconds for 1 c.c.) with the patient lying down Malaria In the above tables for Resochin, some of the tablets may be replaced by ampoules. One tablet is equivalent to one ampoule. Adults should not receive more than 2 ampoules at once, children under 10, not more than 1 ampoule, infants not more than half an ampoule. These amounts can be repeated on the same day, if necessary. A transfer to oral treatment should be made as soon as possible Amoebic Hepatitis and Liver Abscess In severe cases give on the first day 1—2 ampoules additionally to the Resotren treatment described below
Packings:	5 ampoules of 5 c.c
Resotren 7-chloro-4-(4-diethylamino-1'-methylbutylamino)-quinoline-di-7-iodo-8-hydroxyquinoline-5-sulphonate	Intestinal Amoebiasis (acute and chronic amoebic dysentery) Extraintestinal Amoebiasis (amoebic hepatitis and liver abscess) In mild sub-acute and chronic amoebiasis and in mild liver involvement, adults 3 x 1 tablet daily for 8 days, then 2 x 1 tablet daily for 8 days In acute dysentery and hepatitis with threatening necrosis, the above dosage is raised from the 2nd-4th day inclusive to 3 x 2 tablets After-treatment, if necessary, may be 1 tablet daily for 8—10 days for mild cases, and 2 tablets daily for the same period for severe cases For very severe cases, an intensive course may be given. This consists of 60 tablets in 14 days: 1st day 3 x 1 tablet; 2nd-7th day, 3 x 2 tablets; 8th—14th day 3 x 1 tablet Tablets should never be taken on an empty stomach, but always at the end of a light meal For children doses are approximately as follows: 9—12 years: two-thirds adult dose. 5— 8 years: one-half adult dose less than 5 years: one-quarter to one-third adult dose.
Packings:	20 and 300 tablets of 0.5 g

Solustibosan

37 % solution of pentavalent antimony gluconate. The solution contains 100 mg antimony^{V} per c.c.

Kala-azar

Adults and children receive intramuscularly or intravenously 0.1—0.15 c.c./kg bodyweight daily. The injection may be given as one dose or divided into a morning and evening dose. In Mediterranean kala-azar a total of 10 daily doses should be given. In resistant cases the above course should be repeated.

Oriental Boil

When only single boils are present they should be infiltrated with Solustibosan. If the boils are numerous, and in mucosal leishmaniasis, systemic treatment as for kala-azar. If necessary, combine local and systemic treatment. Total daily dose of Solustibosan by all routes, not more than 0.15 c.c. kg. bodyweight.

Packings: 10 and 50 ampoules of 3 c.c.
Bottles of 60 c.c.

Supranol

Sulphonamide complex consisting of an equimolecular combination of 2 (4'-aminobenzene-sulphonamido)-4-methylpyrimidine and the 4 aminobenzene sulphothiocarbamide salt of 4-aminomethyl-benzenesulphonamide

Plague, Smallpox, Lymphogranuloma venereum, Tropical Ulcer

In severe cases the following doses should be given for the first 2—3 days. This dosage may be given by the oral or intravenous route, and should be divided into 3—5 single doses.

Age	Maximum daily dose	
	Tablets	Ampoules
Infants		
Up to 3 months	2	½
Up to 6 months	3	½—1
Up to 12 months	4	1
Children 1—3 years	4—6	1—1½
Children 4—6 years	4—6	1—1½
Over 7 years	6—8	1½—2
Adults	10—12	2½—3

Name and Composition	Administration and Dosage
Solu-Supronal 20 % aqueous solution of Supronal 1 ampoule contains 2 g. of effective substance. 1 bottle of 50 c.c contains 10 g of effective substance.	**Plague, Smallpox, Lymphogranuloma venereu Tropical Ulcer** (continuation) In adults the commencing dose should not be l than 5—6 g daily. As far as tolerability is conce ed, the dose may be raised to 10 g daily witho hesitation when severe infections necessitate this From the 2nd—3rd day on the doses given in th table may be reduced by 20—25 %, if the clinic picture permits. If Supronal treatment is continued for longer than one week, a regular blood and urine control should be made. The tablets should not be taken on an empty stomach When the maximum doses are being given, in order to improve the excretion of the drug, enough sodium bicarbonate should be administered to make the reaction of the urine alkaline Supronal should, when given intravenously, be injected slowly at body temperature. The paravenous injection of Supronal should be avoided. Subcutaneous and intramuscular administration is contraindicated because of the high concentration of the solution
Supronal Eye-Ointment	**Trachoma** Supronal Eye Ointment (10 % Supronal) is squeezed into the conjunctival sac once or several times daily.

Packings: 10, 20, 250, and 1,000 tablets of 0.5 g
5 and 25 ampoules of 10 c.c.
Tubes of 5 g of Eye-Ointment.

ame and Compos tion	Administration and Dosage
Yatren 75 % 7-iodo-8-hydroxyquinoline-5-sulphonic acid plus 25 % $NaHCO_3$. Yatren pills are prepared from this combination	**Ameobic dysentery** Adults 3 x 1 pill daily after meals, increasing to 3 x 3—4 pills daily. Duration 7 days, then every other day or every third day repeat dose, giving a four day rest period in between for three weeks. Total dose 80—100 pills. Children 3 x ½—2 pills, whole or crushed in milk or food. Duration of course as for adults. The laxative action of Yatren is a part of the treatment. If the diarrhoea is, initially, too marked, the dosage should be reduced until it subsides. In chronic and severe cases, and in colitis, oral administration should be supplemented with rectal, using enemas with a 0.5 % Yatren solution. Adults commence with 200 c.c. increasing to 600—800 c.c. daily for 5—7 days, then every second day. With Yatren pills in the intervals, dosage as above; Total enema dosage 20—25 g. Children 15—160 c.c. with a frequency, duration and combination with Yatren pills, as above. The enema should be at body temperature and retained for 6—8 hours. If retention of the enema is found to be difficult at first, 20—30 drops of Tinc. opii (children less) should be added to the Yatren. A preliminary cleansing enema is important. In preparing the solution the instructions given in the packing should be strictly adhered to. The solution should never be boiled — since this breaks down the Yatren into toxic degradation products.
Packings	25, 50, and 100 pills of 0.25 g Bottles of 10 g

[illegible]